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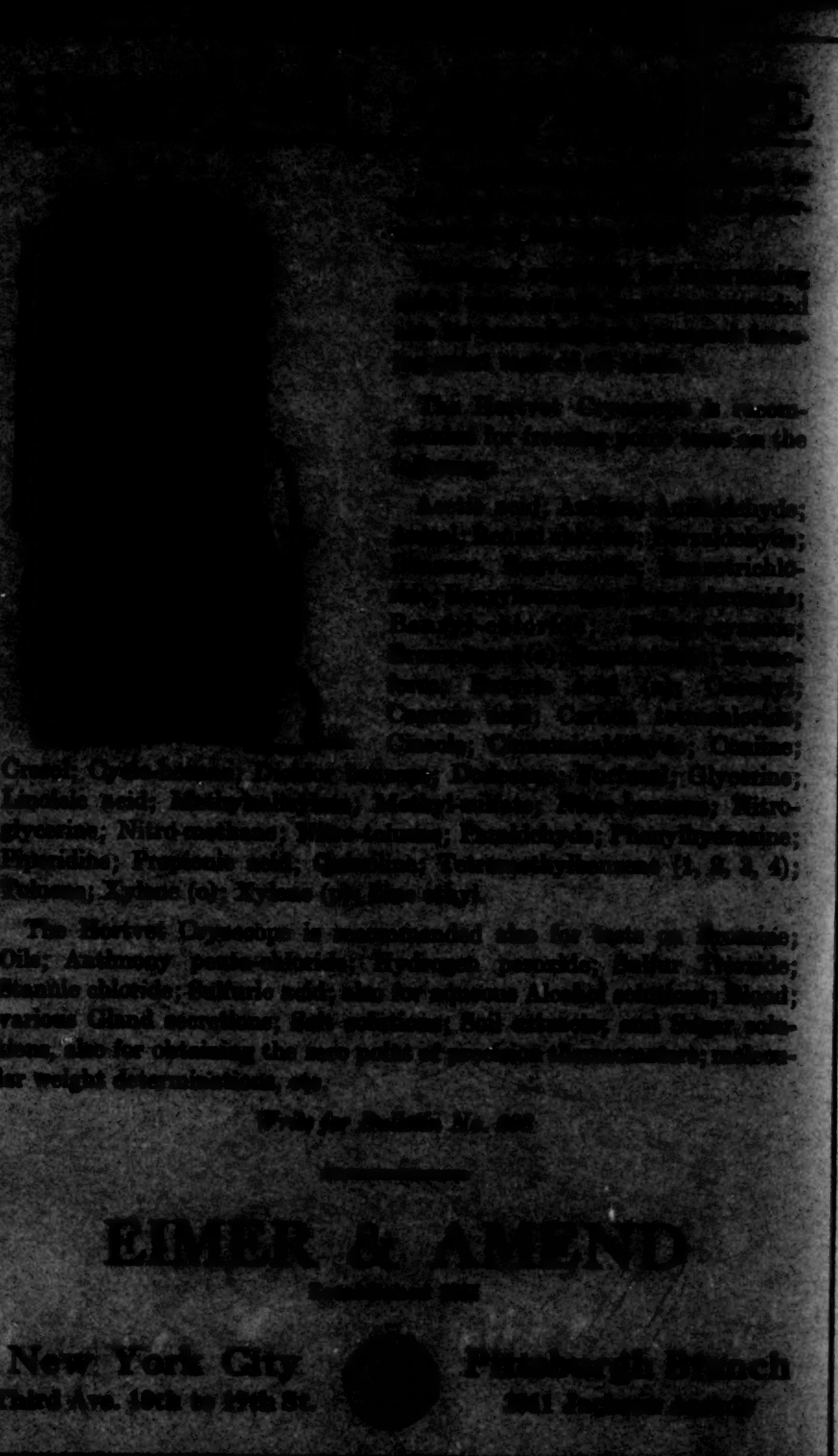

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THE REMOVAL OF AMMONIA FROM URINE PREPARATORY TO THE DETERMINATION OF UREA.

By GUY E. YOUNGBURG.

(*From the Laboratory of Biological Chemistry, Medical Department, University of Buffalo, Buffalo.*)

(Received for publication, November 27, 1920.)

In the determinatian of urea by the urease method of Marshall (1) as modified by Van Slyke and Cullen (2) the ammonia determination can be eliminated by preliminary removal of ammonia by the permutit method of Folin and Bell (3). It is evident that such a simple and rapid process should be very desirable in place of the present complete ammonia determination required whenever urea is determined.

The more satisfactory and less expensive urease solution of Folin and Youngburg (4) may also be used instead of the acetone-insoluble preparation of Van Slyke and Cullen. The latter does not go into solution well and in the course of a day is largely precipitated, forming a muddy viscous mixture-solution which is rather undesirable to use, especially in aeration.

Method for Removal of Ammonia.—Dilute 5 cc. of the urine to 50 cc. (10 to 50 if very dilute urine) and mix well. Place 3 to 4 gm. of dry permutit in a wide bottom flask, preferably a 200 cc. or 250 cc. volumetric, and add 20 to 25 cc. of the diluted urine. Agitate for 5 minutes. Allow to settle 15 to 30 seconds and then pour through a thin filter paper.[1] If there is no permutit "dust" the urine may be decanted without filtering. The ammonia is completely removed.

Determination of Urea.—The Van Slyke and Cullen method is slightly modified to use the alcoholic urease solution and the filtrate from the permutit-urine mixture. To 5 cc. of the filtrate

[1] Filter paper must not contain any appreciable amount of ammonia. It was found during the work reported here that some filter papers contained as much as 1 to 2 mg. of ammonia N.

2 cc. of the alcoholic urease solution of Folin and Youngburg are added, together with 2 drops of a buffer solution, which contains Na_2HPO_4 and NaH_2PO_4, each in molecular concentration (142 and 120 gm. respectively per liter). 15 minutes are allowed for decomposition of the urea by the enzyme. The remainder of the determination is carried through as described by Van Slyke and Cullen.

TABLE I.

Effect of Increased Amounts of Ammonia.

Urine A.

	mg. per cc.
Total N	8.43
Urea N	7.26 (86.10 per cent of total N).
Ammonia N	0.41 (4.86 " " " " N).

To 10 cc. portions of the urine were added different amounts of ammonia N [$(NH_4)_2SO_4$] and the volume was made up to 50 cc. according to the usual dilution in urea determination. After agitating with permutit in the proportion of 6 gm. to 30 cc. of the diluted urine, the total N was determined on a 50 cc. portion and urea on a 5 cc. portion.

Sample No.	Ammonia N added.	Total N obtained.	Urea N obtained.	
	mg.	*mg. per cc.*	*mg. per cc.*	*per cent**
1	0	8.11	7.22	85.69
2	5	7.95	7.17	85.05
3	10	8.02	7.14	84.70
4	15	8.09	7.17	85.05
5	20	8.02	7.21	85.36
6	25	7.95	7.36	87.35
7	30	8.01	7.34	87.02
8	35	7.97	7.34	87.02
9	40	7.99	7.20	85.36
10	45	8.06	7.28	86.36
11	45 + 250 mg. NaCl.	8.12	7.34	87.02

* Total N = 8.43 mg. per cc.

The data from the experiments described in Tables I and II show that urea is not absorbed or retained in any way by permutit, also that the urinary salts even in somewhat more than the maximum amounts found in urines (undiluted) are without effect.

In Table I there have been added increasing amounts of ammonia N up to 45 mg. per 10 cc. of urine. This amount is more than has been found in urines. The total N and urea N

determinations, however, show that all the ammonia N was removed by the permutit.

In Table II ammonia N was added so that the urine contained 13.35 per cent of its N in the form of ammonia. Increasing amounts of NaCl were added, also a mixture of ordinary urinary

TABLE II.

Effect of Increased Amounts of Salts.

Urine B.

	mg. per cc.
Total N	9.86
Ammonia N	1.0
NaCl	19.4

Ammonia N [$(NH_4)_2SO_4$] and other salts were added to the urine and dilution was made as shown below. The urine was then agitated for 5 min. with permutit and filtered and the total N determined on 10 cc. of the filtrate (representing 4 cc. of the undiluted urine).

Sample No.	Urine taken.	NH_3 N added.	NaCl added.	Water.	Total N calculated to undiluted urine.	Ammonia N removed.	
	cc.	mg.	mg.	cc.	mg. per cc.	mg. per cc.	per cent of total N*
1	20	7.3†	200	50	8.89	1.335	13.05
2	20	7.3	400	50	8.85	1.375	13.45
3	20	7.3	800	50	8.79	1.435	14.03
4	20	7.3	1,600	50	8.94	1.285	12.47
5	20	7.3	3,200	50	8.82	1.405	13.64
6	20	7.3	6,400	50	8.79	1.435	14.03
7	20	7.3	8,000	50	8.89	1.335	13.05
8	20	7.3	1,600 + 540 $NaH_2PO_4.4H_2O$ + 500 $Na_2SO_4.10H_2O$.	50	8.80	1.425	14.03

* Total N = 9.86 mg. 0.365 mg. per cc. added = 10.225 per cent.

† Through a miscalculation this amount of N makes the total ammonia N 13.35 per cent instead of 10 per cent as intended. 7.3 mg. in 20 cc. = 0.365 mg. per cc.

salts in amounts exceeding those found normally or pathologically. The results show that the ammonia was removed in all cases.

Urines A and B were normal urines of acid reaction. Total N was determined by the simplified macro-Kjeldahl method of Folin and Wright (5). Urea was determined by the Van Slyke

and Cullen method except that alcoholic urease solution was used. Ammonia was determined by the permutit colorimetric method (3) and chlorides by the usual Volhard method.

The removal of ammonia by permutit may of course be employed equally well in any of the methods for urea determination. The writer has employed it especially for the determination of urea by direct Nesslerization (4). It is particularly desirable in that case because no aeration is employed.

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CHEMICAL STUDY OF SEVERAL MARINE MOLLUSKS OF THE PACIFIC COAST.*

By P. GERHARD ALBRECHT.

(From the Department of Chemistry and Hopkins Marine Station of Stanford University, Palo Alto.)

(Received for publication, December 20, 1920.)

The marine invertebrate forms of animal life have not often been the subject of chemical investigation. Lacking, as a class, the greater freedom of movement and the more highly organized nervous and circulatory systems which the possession of a bony skeleton allows, these forms, in general, are more sluggish in their metabolic activities, less responsive to chemical influences, and less attractive subjects of chemical study.

Among the mollusks, the oyster has been the subject of considerable scientific investigation in regard to its composition, food value, and commercial propagation. The clam and *Abalone* have in recent years, particularly on the Pacific Coast, risen to a position of considerable economic importance, and regulations limiting the size of the animals collected for commercial use and the season of the year during which they may be taken are now in force.

The following investigations deal specifically with five distinct species of marine mollusks which occur in considerable abundance along the coast of California. These are *Abalone*, Pismo clam, *Cryptochiton*, *Ischnochiton*, and owl limpet.

1. Abalone.—*Abalone* belongs to the family of marine snails, the Haliotidæ. It appears to be exclusively herbivorous in its habits, feeding chiefly on kelp and sea lettuce. The animals can often be obtained at low tide, as they occur on the rocks at depths varying from 1 to 30 meters below mean sea level. When full

* This paper is a part of a thesis to the Department of Chemistry of Stanford University in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

grown they weigh from 0.5 to 2 kilos. For commercial purposes *Abalone* on this coast is usually obtained by Japanese divers, who, equipped with diving suits, work from boats at some distance off-shore at depths up to 30 meters.

2. Pismo Clam, Tivella stultorum.—This bivalved mollusk occurs along the ocean shore imbedded in mud and sand at depths of from 1 to 4 feet. Its length is from 1 to 4 inches, depending on the age of the animal and the environment in which it grows. Clams, as all other lamellibranchs or bivalves, feed chiefly on diatoms carried by the brackish tidal currents.

3. Chitons, Coat-of-Mail Shells.—The chitons are an ancient family, biologically speaking, ranking among the most primitive of the molluscan forms. The shell is composed of eight separate but overlapping plates, forming a dorsal shield, and so giving the specimen the form of a boat embedded in a leathery girdle of skin. Underneath these plates are found the organs of the animal essential for its life and below these a muscular foot. Two chitons obtainable in quantity on the Pacific Coast were studied. They are:

Cryptochiton stelleri, the giant chiton.

Ischnochiton conspicuus, the *Ischnochiton.*

Cryptochiton is the largest of the family of chitons, and is readily obtained at low tide clinging loosely to rocks or lying in tide pools. It feeds on the marine vegetation of that zone, and frequently upon sand with attached microscopic particles. Its weight is from 0.5 to 1.5 kilos.

Ischnochiton is much smaller in size, weighing from 30 to 50 gm.

4. Owl Limpet, Lottia gigantea.—This form is small in size, the oval shell averaging from 5 to 6 cm. in length. It feeds chiefly on diatoms and seaweed.

The material for this investigation was secured in abundance at various points along the coast of California. The *Abalones* were obtained chiefly in Monterey Bay, near Carmel, Point Lobos, Point Sur, and the bay around La Jolla in southern California. At times when unfavorable weather made shore collecting impossible, specimens were secured from Japanese divers. *Cryptochitons* were gathered at low tide from the rocks or tide pools of Half Moon Bay and near the shore at Carmel

Beach. The Pismo clams were obtained from Pismo Beach, where the beaches are plowed at certain seasons for a large commercial yield. Special care was taken to obtain fresh, uninjured material. The tissues and organs studied were the muscle, liver, reproductive system, and blood under normal, and in some cases under fasting conditions. Material not used in a fresh condition was preserved with alcohol.

The Chemical Investigation.

Digestive Enzymes in Stomach and Intestines.

The first investigations on this subject on various land and marine mollusks were carried on by Bernard (1). He found that the acid fluid in the intestines of *Loligo*, *Limax*, and *Ostrea* digests starches and fats. The same fact was established later by Yung (2) on the stomach juice of *Helix*, a common land mollusk. Biedermann and Moritz (3) found that during fasting periods the fluid of the stomach and intestines of snails is free from sugar, but contains a strong starch-splitting enzyme and is also able to invert cane-sugar. At the same time they discovered the presence of cytase in the secretion of the liver which had passed into the intestine, while the extract of the liver itself was inactive. The experiments of Biedermann and Moritz were confirmed by Müller (4) in 1900. Fredericq (5) showed that a proteolytic enzyme is present in the intestinal fluid of *Arion rufus* which acts in weakly alkaline but not in acid solution, while in *Mytilus* this fluid is active in acid solution as well. De Bellesme (6) established further the presence of a tryptic enzyme in the secretion of the *Octopus* liver. Examining the intestinal contents of *Aeolis*, Krukenberg (7) found also a peptic enzyme. Since then Abderhalden and Heise (8) have shown that proteolytic enzymes are present in the alimentary canal of many invertebrates.

Sellier (9) reported a proteolytic enzyme and Bernard a lipase in the gastric secretion of a few marine invertebrates. Mendel and Bradley (10) found in their study of *Sycotypus* that digestion in this mollusk is affected by the secretion of two different kinds of glands: the salivary glands, containing a proteolytic enzyme with amphoteric action in the cold, resembling trypsin; and the liver and hepatopancreas glands, elaborating enzymes which hydrolyze carbohydrates and fats.

In the present investigation tests were carried out on glycerol extracts for the following enzymes employing the usual methods: amylase, cytase, emulsin, glycogenase, lactase, lipase, maltase, pepsin, sucrase, trypsin, urease, uricase.

It was found that a temperature of 37°C. was satisfactory for the reactions involved. The results are presented in Table I.

TABLE I.

Enzymes of the Digestive Tract.

	Abalone.	Pismo clam.	*Crypto-chiton.*	*Ischno-chiton.*	Owl limpet.
Amylase	+	+	+	+	+
Cytase	−	−	−	−	−
Emulsin	+	+	+	+	+
Glycogenase	+	+	+	+	+
Lactase	+	+	+	+	+
Lipase	+	+	+	+	+
Maltase	+	+	+	+	+
Pepsin	+	+	+	+	+
Sucrase	+	+	+	+	+
Urease	+	+	+	+	+
Uricase	−	−	−	−	−

The Muscle.

The most important contractile tissue of *Abalone*, the chitons, and the limpet, in point of size, is the large pedal muscle, occupying the open end of the shell and when extended projecting some distance beyond it. This tissue makes up approximately half the body mass exclusive of the shell in the last two forms and considerably more than half in *Abalone*.

Among invertebrates very few investigations of muscle tissue have dealt with molluscan forms. The most favored animal in this respect seems to be *Octopus*. Other marine animals studied regarding muscle extractives are *Mytilus edulis, Pecten opercularis, Venus mercenaria, Sepia, Sycotypus, Fulgur, Ostrea,* and *Haliotis*.

Glycocoll was found in the adductor muscle of *Pecten irradians* by Chittenden (11) in quantities from 0.39 to 0.71 per cent. Tyrosine and leucine, decomposition products of proteins, were reported by Dohrn (12) in crab muscle, further by Krukenberg (13) in the muscle of the lobster, but the occurrence of free tyrosine in any quantity in the muscle of living mollusks is by no means established.

Of the inorganic constituents, special importance has been attached to iron as one of the essential constituents of the invertebrate muscle. Schneider (14) made a special study on iron in muscle, liver, and mantle of mollusks. Giunti (15) and later Dubois (16) determined the quantity

of copper present in lower animals. A complete inorganic analysis has been carried out by Henze (17) on *Octopus* and by Meigs (18) on *Venus mercenaria*, a clam occurring on the eastern coast of this country.

Methods of Analysis.

Muscle tissue of *Abalone*, Pismo clam, *Cryptochiton*, *Ischnochiton*, and owl limpet was examined for the following components: water, total solids, ash, alcohol-soluble portion, ether-soluble portion, proteins, purines, creatine, creatinine, uric acid, glycogen, inorganic constituents, and enzymes, employing in the preliminary treatment in the main the methods of Koch and Carr (19). The alcoholic extract was examined for total solids, ash, total nitrogen, proteose nitrogen, urea nitrogen, ammonia, total sulfur, total phosphorus, inorganic sulfur, inorganic phosphorus, creatine, creatinine, uric acid, and reducing sugars. The analysis of the ether-soluble portion included total solids, total nitrogen, total sulfur, and total phosphorus, while that on the alcohol- and ether-insoluble portion included total nitrogen, protein, total sulfur, total phosphorus, phospho-protein phosphorus, uric acid, creatine, creatinine, and reducing sugars.

Ammonia was determined by Folin's method, based upon the absorption of free ammonia in acid and the titration of the excess of acid. Urea was estimated by Marshall's urease method (20), and later checked by the method of Van Slyke and Cullen (21). Creatinine and creatine were determined by the methods of Folin and uric acid by that of Benedict and Hitchcock.

The results of these analyses are given in Table II.

The only positive tests for enzymes in muscle extracted with glycerol were an amylase and glycogenase in *Cryptochiton*, and a urease in the Pismo clam.

DISCUSSION.

The water content of the muscle tissue is higher in gastropods and lamellibranchs than in mollusks not very closely related to them, as Table III indicates.

No records are available regarding the content of fat in the muscle of mollusks. Apparently it may vary widely among different species. *Ischnochiton* carries larger amounts of fat than

TABLE II.

Constituents.	*Abalone.*	Pismo clam.	*Crypto-chiton.*	*Ischno-chiton.*	Owl limpet.
100 gm. of fresh muscle tissue.					
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Water	70.90	76.40	75.10	70.50	73.80
Total solids	29.10	23.60	24.90	29.50	26.20
Ash	3.42	5.10	3.52	3.88	7.54
Alcohol extractives, F1 + F2	8.59	8.04	4.01	12.53	7.34
Lipin fraction, F1	1.56	0.99	1.00	5.84	0.30
Water-soluble fraction, F2	7.03	7.05	4.05	6.69	7.04
Alcohol- and water-insoluble fraction, F3	20.5	15.57	20.93	16.94	18.83
Ash of F1 + F2	1.22	1.20	1.30	1.48	1.74
" " F3	2.20	3.90	2.22	2.40	5.80
Total N	3.61	1.61	3.55	2.70	3.19
Protein	23.00	10.26	22.60	17.27	20.30
Purine N	0.06	0.056	0.94	0.083	Not determined.
Creatine and creatinine	0.01	Trace.	Trace.	Trace.	Trace.
Uric acid	None.	None.	None.	None.	None.
100 gm. of F1 + F2 + F3.					
Alcoholic extracts, F1 + F2.	29.50	34.10	16.10	42.50	28.00
Alcohol-insoluble residue, F3	70.50	65.90	83.90	57.50	72.00
100 gm. of F1 + F2.					
Total solids, F1	17.90	12.30	25.00	46.00	4.10
" " F2	82.10	87.70	75.00	54.00	95.90
Ash of F1 + F2	17.35	16.90	13.20	22.10	24.70
" " F3	11.00	25.00	10.50	14.10	30.70
100 gm. of F1.					
Total N	6.46	5.20	0.40	1.45	15.0
Amino-acid N.	0.227	0.270	0.390	0.127	1.020
Total sulfur	1.03	1.00	1.50	0.51	3.67
" phosphorus	1.08	1.83	1.68	0.48	1.05
Lipin sugar	None.	None.	None.	None.	None.

TABLE II—*Concluded.*

Constituents.	*Abalone.*	Pismo clam.	*Cryptochiton.*	*Ischnochiton.*	Owl limpet.
		100 gm. of F2.			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Total N	6.40	8.64	16.2	6.75	6.16
Amino-acid N	3.07	3.60	5.51	0.99	2.75
Proteose N	0.34	0.34	1.08	0.45	0.41
Urea N	0.16	0.06	None.	0.12	0.04
Urea	0.35	0.14	"	0.30	0.09
Ammonia	0.35	0.35	0.89	0.37	0.36
Total sulfur	6.55	5.65	7.2	7.17	6.23
Inorganic sulfur	0.085	0.1	1.0	0.28	0.17
Total phosphorus	0.49	0.53	1.41	0.41	0.19
Inorganic phosphorus	0.043	0.175	0.360	0.037	0.082
Creatine and creatinine	Trace.	None.	None.	None.	None.
Uric acid	None.	"	"	"	"
Reducing sugars on hydrolysis	14.4	27.2	9.67	9.58	5.26
		100 gm. of F3.			
Total N	13.23	10.81	14.73	14.06	12.80
Protein (× 6.37)	82.80	67.70	92.20	88.01	80.13
Total phosphorus	0.42	0.53	0.19	0.40	0.52
Phospho-protein phosphorus	0.47	0.78	0.64	0.47	0.46
Total sulfur	0.69	0.86	0.97	0.58	1.03
Creatine and creatinine	Trace.	None.	None.	None.	None.
Uric acid	None.	"	"	"	"
Reducing sugars after hydrolysis	10.40	19.24	0.25	4.10	0.64
		Inorganic constituents per 100 gm. of tissue.			
SiO_2	1.24	0.66	1.57	2.31	2.34
Fe_2O_3	1.35	1.56	1.21	1.20	1.72
CaO	0.31	0.85	0.47	1.05	0.46
MgO	0.29	0.43	0.42	0.40	0.42

Abalone and the bivalved clam. The lowest is the owl limpet, which anatomically is more closely related to *Abalone* than to *Cryptochiton* or *Ischnochiton*. The muscle of *Abalone*, limpet, and *Cryptochiton* contains relatively large amounts of combined nitrogen, which is an approximate measure of the total protein. The

muscle of *Ischnochiton* and Pismo clam, in the order named, runs notably lower in these constituents.

Determinations for total nitrogen were extended to the sexes in the case of *Abalone*, *Cryptochiton*, and *Ischnochiton*, but the results show no substantial difference between them.

TABLE III.

Animal.	Water.	Total solids.	Ash.	Reference.
	per cent	*per cent*	*per cent*	
Octopus (muscle)	77.5	22.70		Henze (17).
Mytilus edulus (total animal)	82.20	17.80		Voit (22).
Ostrea (" ")	80.50	19.50		Balland (23).
Astacus fluviatilis (" ")	77.11	22.89	9.061	von Bezold (24).
Venus mercenaria (" ")	74.44	25.56	1.46	Meigs (18).
Haliotis (muscle)	70.90	29.50	3.42	Albrecht.
Tivella stultorum (muscle)	76.40	23.60	5.10	"
Cryptochiton (")	75.10	24.90	3.52	"
Ischnochiton (")	70.50	29.50	3.88	"
Lottia gigantea (")	75.80	26.20	7.54	"

TABLE IV.

Animal.	Purine N.	Reference.
	per cent	
Octopus	0.0456	Henze (17).
Vertebrates	0.055–0.071	" (17).
Abalone	0.06	Albrecht.
Pismo clam	0.056	"
Cryptochiton	0.094	"
Ischnochiton	0.083	"

The purine nitrogen content of the muscle of these mollusks is small, yet relatively large as compared with the corresponding tissue in *Octopus* and the vertebrates, as Table IV indicates.

Uric acid was not present in the muscle tissue of the species examined, and in fact it has never been reported in the muscle of mollusks. Creatine and creatinine were found in appreciable quantities only in *Abalone* muscle. The absence of creatinine in the muscle of the chitons, limpet, and clam was confirmed by the addition and accurate *redetermination* of known amounts of

creatinine. This discovery of creatine and creatinine in *Abalone* muscle is of particular interest, as it is the first time these two substances, which are among the most important metabolic products in the vertebrates, have been found in the muscle tissue of mollusks.

In *Abalone*, Pismo clam, and *Ischnochiton*, the ammonia content is almost the same. The large amount of lipins in *Ischnochiton* is of especial interest. It is approximately four times the amount found in *Abalone*, six times that in *Cryptochiton* and the Pismo clam, and nineteen times that in the owl limpet. Mammalian muscle contains about 2 to 3 per cent of lipins. The Pismo clam, not especially high in its glycogen content, leads in reducing sugars. No results on other marine animals, besides those examined in the course of this investigation, are available for comparison.

The large amount of iron is worthy of note. Former analyses carried out by Henze on *Octopus* and by Meigs on the clam show no iron or only traces. In the mollusks examined by the author it is almost the same in amount but notably high in the various forms.

Calcium which is present in all mammalian and invertebrate tissue was also determined, the largest amount being found in *Ischnochiton* while *Cryptochiton*, anatomically closely related, carried only a very small quantity. The other closely related forms, *Abalone* and owl limpet, contain almost the same amount of calcium. Magnesium does not vary much in the mollusks examined. Potassium and sodium were not determined.

Very few enzymes are present in the muscle of mollusks. Careful estimations gave as a rule negative results. This is astonishing at first, since, having found large amounts of glycogen and fat in the muscle tissue, we might expect to find the corresponding enzymes. An article by Bradley and Kellersberger (25) leads, however, to the general conclusion that tissues rich in glycogen are poor in diastatic enzymes, and, conversely, those poor in glycogen are rich in diastatic enzymes. This is practically what was found in this investigation.

It is possible that in an animal, the whole metabolism of which is on so low a level, an amount of enzyme too small to be detected is still sufficient to hydrolyze the glycogen stored in the pedal

muscle. The same condition seems to hold in the case of lipase, the fat-splitting enzyme, as Bradley has shown for higher animals. Some of the most active fat-producing tissues are poorer in lipase than many which never contain or produce more than a small percentage of fat. Experiments carried out with a glycerol extract of muscle of *Abalone*, Pismo clam, *Cryptochiton*, *Ischnochiton*, and owl limpet gave striking examples which support this general view. An exception was noticed in *Cryptochiton* muscle on diastase, and in Pismo clam on urease.

SUMMARY.

The above investigation has dealt with the chemical composition of five species of marine mollusks occurring in abundance along the coast of California. These are *Abalone*, Pismo clam, giant chiton, *Ischnochiton*, and owl limpet, of which the first two are of rapidly growing importance as food for human consumption.

1. The digestive juices of the stomach and intestines of each of these forms have a distinct acid reaction and are notably rich in enzymes. A catalase, glycogenase, lactase, lipase, maltase, protease, invertase, urease, emulsin, and an amylase gave pronounced reactions. A cellulose-digesting enzyme was not found in any case.

2. A complete analysis of the muscle tissue was made. Particularly notable among the results on this tissue are the relatively high protein content of *Abalone*, 23 per cent; the low protein content of the Pismo clam, 10.26 per cent; the large amount of ash in all the forms; and especially in the limpet 7.54 per cent and the Pismo clam 5.1 per cent as against an average of approximately 1 per cent in the common mammalian muscle tissues used as food and 1 to 1.5 per cent in the sea fishes; the presence of determinable amounts of urea in *Abalone*, Pismo clam, *Ischnochiton*, and limpet; the large amounts of reducing sugars yielded on hydrolysis of the alcoholic extract from 5.26 to 27.2 per cent of the residue after removal of the alcohol; and finally the occurrence of creatinine and creatine in *Abalone*, here reported for the first time in molluscan tissue. Enzymes were found only in three cases: an amylase and glycogenase in *Cryptochiton*, and a urease in the Pismo clam.

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HEMICELLULOSE OF APPLE WOOD.*

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This paper covers the preliminary chemical phase of an investigation upon conditions of composition associated with fruitfulness in the apple and other fruit trees. Roberts[1] has recently presented the general economic aspects of the problem, placing special emphasis upon the relation of the amount of spur growth to fruiting, with a discussion of some external conditions which modify it. The present article deals with the composition of apple spurs and adjacent wood, with special reference to the hemicellulose fraction.

Butler, Smith, and Curry[2] have published data on the proximate composition of different aged branches, trunk, and roots of the apple tree at successive stages, from spring dormancy to leaf fall. These investigators concluded that storage carbohydrates in their material were in the form of sucrose and starch. The most abundant constituent of 1 year branches, aside from crude fiber, was starch. At the time of bud swelling the branches contained over 30.0 per cent of this constituent. At this time also the nitrogen content was greatest in the 1 year branches, amounting to about 1.0 per cent, equivalent to over 6.0 per cent of protein. It should be noted that in the investigation here referred to starch was determined by the method of acid hydrolysis[3] and

* Published with the permission of the Director of the Wisconsin Experiment Station.

The writers are indebted to J. A. Anderson and S. N. Epstein for some of the data presented.

[1] Roberts, R. H., *Wisconsin Agric. Exp. Station, Bull. 317*, 1920.

[2] Butler, O. R., Smith, T. O., and Curry, B. E., *New Hampshire Agric. Exp. Station, Techn. Bull. 13*, 1917.

[3] Correspondence with Dr. O. R. Butler.

therefore includes the hemicellulose compounds, as defined by Schulze.[4] Hooker[5] has made similar analyses of apple spurs, including determinations of the true starch, at successive dates throughout the year. He concludes that fruit bud differentiation occurs as a result of conditions leading to high starch and low nitrogen content.

Analytical.

In the work here reported samples were taken from the trees soon after the dew had evaporated, in an attempt to avoid metabolic changes which might follow the onset of rapid photosynthesis in the leaves. The samples were collected in covered glass jars, weighed as soon as they could be taken to the laboratory, and dried to constant weight at 80°C. The dried material was ground in a drug mill and reduced to a fine powder in a Dreef[6] mill, in which condition it passed through a 100 mesh sieve. Fig. 1 shows the origin of the new growth (*N*), spur (*S*), and base wood (*BB*) samples. It represents the first stage of development of fruiting branches examined.

Moisture was determined by drying 2.0 gm. samples to constant weight at 110°C. *Ether extract* was determined in the residue by extracting for 12 to 14 hours with anhydrous, alcohol-free ether in the Caldwell type of apparatus for continuous extraction. *Sugars* were extracted by boiling the fat-free residue with 100 cc. of 90 per cent alcohol for 1 hour under a reflux condenser. About 0.3 gm. of $CaCO_3$ was added previously, to prevent inversion of disaccharides by acids. After filtering and washing the residue with hot alcohol the extract was evaporated at 60°C., taken up with water, filtered again, and washed to a volume of 100 cc. The reducing sugars in this solution were determined as glucose by the Munson-Walker method.[7] Total sugars were determined as glucose by the Defren-O'Sullivan method,[8] after

[4] Schulze, B., *Landw. Jahrb.*, 1894, xxiii, 1.

[5] Hooker, H. D., Jr., *Univ. Missouri Agric. Exp. Station, Research Bull. 40*, 1920.

[6] Wiley, H. W., Principles and practice of agricultural analysis, Easton, 2nd edition, 1914, iii, 12.

[7] Munson, L. S., and Walker, P. H., *J. Am. Chem. Soc.*, 1906, xxviii, 665.

[8] Defren, G., *J. Am. Chem. Soc.*, 1896, xviii, 751.

boiling for 45 minutes with HCl (1: 10), neutralizing, and diluting to 500 cc.

Starch and *dextrin* were determined by digesting the residue from the extraction of sugars with saliva, after gelatinizing the starch by treatment with boiling water. The resulting sugars and dextrins were washed out, hydrolyzed with acid, and determined by the Defren-O'Sullivan method. *Acid-hydrolyzable* material was determined by boiling the residue from the starch

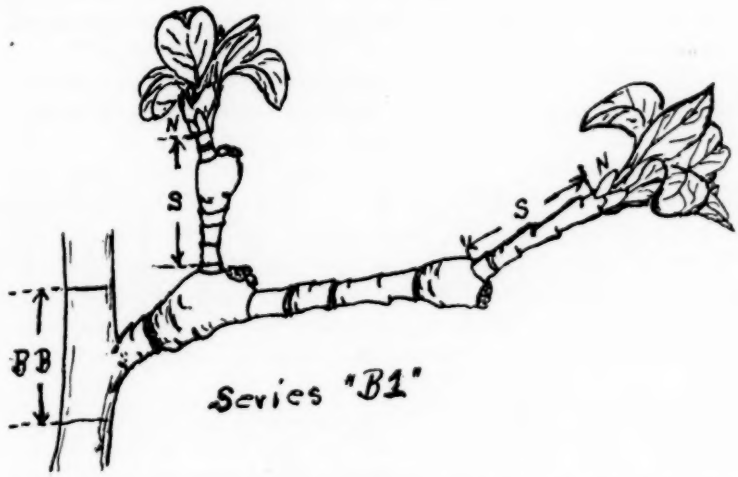

FIG. 1. Tissue taken in sampling apple wood of blossom-bearing branches. *BB*, base wood; *S*, spur; *N*, new growth of spurs.

determination with dilute HCl (1: 10) for 3 hours under a reflux condenser. After filtering and washing, the extract was neutralized and analyzed for glucose by the Defren-O'Sullivan method. *Lignin* was determined by extraction with 72 per cent H_2SO_4, after extraction of the tissue with benzene and alcohol, according to the method of Dore.[9] *Cellulose* was determined by successive chlorinations, after extraction with benzene and alcohol, also according to Dore.[9] *Protein* was calculated by multiplying the total nitrogen by 6.25, the factor commonly used in this connection. The nitrogen was determined by the Gunning-Kjeldahl

[9] Dore, W. H., *J. Ind. and Eng. Chem.*, 1920, xii, 477.

method,[10] with the added use of $CuSO_4$ in the digestion. *Ash* was determined by charring and extracting with water, subjecting the residue to complete incineration, and finally combining the extract, evaporating, and igniting at low heat.

The materials upon which the determinations were made in the case of base wood were composites of samplings on May 3, 12, 28, and June 8 and 24, 1918. In the case of spurs the data are averages of the composition of the samples taken on the above successive dates. The data for new growth of spurs are from one sample taken July 7. All the analytical results are assembled in Table I.

TABLE I.

Composition of Apple Wood in Fruiting Branches, Calculated on the Dry Matter.

	Ether extract.	Reducing sugars.	Other sugars.	Starch and dextrin.	Acid-hydrolyzable material.	Lignin.	Cellulose.	Protein.	Ash.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
New growth of spurs.	1.62	0.94	5.89	4.87	21.75				
Spurs.....	1.40	4.13	3.77	3.27	27.90			5.36	
Base wood.....	1.76	1.50	2.07	3.50	28.70	18.60	36.50	2.37	3.90

Examination of the analytical data shows that while the acid-hydrolyzable material is very abundant in both spurs and base wood, the true starch and dextrins form only a small percentage of the total weight. The results from a similar analysis of plum wood, in which dextrins were extracted with water before digesting the starch, showed that the percentages of these two constituents were about equal in that material. In the case of the complete analysis of base wood the data form a total of 98.90 per cent.

In view of the probable abundance of pentosans in the wood, determinations of these were made by the method of conversion to furfurol and precipitation with phloroglucin, as modified by Kröber.[11] The result for the base wood was 19.0 per cent. In

[10] Gunning, J. W., *Z. anal. Chem.*, 1889, xxviii, 188.

[11] Kröber, E., *J. Landw.*, 1901, xlviii, 357.

the case of the products recovered from acid hydrolysis of the base wood, after these had been subjected to fermentation with ordinary yeast to remove the hexoses, the result was 10.1 per cent, based also upon the original wood. From these relations it appeared probable that in the acid-hydrolyzable fraction of the wood we were dealing primarily with a mixture of hexose and pentose sugars.

Examination of the Products from Acid Hydrolysis.

This examination is confined to the base wood. A determination of mannan by the method of Schorger[12] gave negative results.

For the preparation of a quantity of the products of hydrolysis 100 gm. of wood were extracted successively with ether and 90 per cent alcohol, and digested free from starch with saliva.. 1 liter of dilute H_2SO_4 (2.5 per cent by volume) was added and the mixture was boiled for 2 hours under a reflux condenser. After filtering and washing, the filtrate was digested with $BaCO_3$ until neutral. The $BaSO_4$ thus produced was filtered off and the solution was made faintly acid with H_3PO_4. On concentrating under a pressure of about 130 mm. at 45–50°C. a thick syrup remained.

This syrupy product was extracted for some time with about 200 cc. of boiling 99 per cent alcohol. The extract was filtered and concentrated *in vacuo* as before. It was then taken up with a little water and decolorized by boiling with norite. After filtering off the norite, the solution was concentrated as before and dried at 44° under a pressure of 127 mm. The final product was 9.9 gm. of a very viscous, pale brown solid.

The residue from extraction with 99 per cent alcohol was subjected to a similar extraction with 95 per cent alcohol, but the amount of material extracted was very small. The product was therefore combined with the previous fraction, and its amount is included therein.

The residue from the alcoholic extractions was taken up in about 35 cc. of cold water. A small amount of pale brown residue was filtered off and the soluble material was recovered and dried as in the case of the alcohol-soluble fraction. There were

[12] Schorger, A. W., *J. Ind. and Eng. Chem.*, 1917, ix, 748.

recovered 2.4 gm. of a solid which was paler and more brittle than that previously recovered.

The specific rotatory power, reducing power, and pentose content of these two fractions of the hydrolysis products were determined. The galactose content of the alcohol-soluble fraction was also determined, through oxidation to mucic acid by the procedure of Dore.[9] All these results appear in Table II.

TABLE II.

Properties of Products from Acid Hydrolysis of Apple Wood.

	Specific rotation.	Reducing power, in equivalents of glucose.	Pentose content.	Galactose content.
	degrees	*per cent*	*per cent*	*per cent*
Alcohol-soluble fraction	26.1	70.0	58.6	0.98
Water-soluble "	27.0	25.1	29.2	

Special attention was given to identifying the sugars in the alcohol-soluble fraction of the hydrolytic products. Tests of the osazones prepared in the usual way with phenylhydrazine hydrochloride showed a high degree of solubility in boiling water, indicating a high percentage of pentose sugars. Fractional preparations of the osazones taken after periods of 5, 10, and 20 minutes in the water bath, when compared under the microscope with osazones freshly prepared from glucose, galactose, xylose, and arabinose, showed a preponderance of either glucose or fructose in the first fraction, while the pentoses predominated in the later fractions. A few crystals which appeared to be galactosazone were observed in the last fraction. Both the Seliwanoff test for ketoses and the osazone test by means of methylphenylhydrazine, as directed by Neuberg,[13] were negative, thus establishing the absence of fructose.

As with the wood itself, a test for mannose as hydrazone by the use of phenylhydrazine gave a negative result. By the absence of other sugars which give the glucosazone test, the presence of glucose as the chief hexose sugar in our preparation is made certain. The absence of arabinose was proved by nega-

[13] Neuberg, C., *Ber. chem. Ges.*, 1902, xxxv, 960.

tive results from the test with diphenylhydrazine according to Neuberg.[14] It is apparent, therefore, that the pentose of our preparation is xylose.

While it is not possible to separate sharply a mixture of sugars of this sort, it is possible to use the analytical data in a comparative way by which the approximate proportions may be ascertained. On the basis of the pentose determination of Table II, and as a result of the identification of sugars, it appears that the alcohol-soluble fraction resulting from hydrolysis consisted of about 58 per cent of xylose. Allowing 1 per cent for galactose, on the basis of the mucic acid test, there would remain 41 per cent of glucose. The specific rotation of a mixture of *l*-xylose, *d*-glucose, and *d*-galactose in these proportions should approximate 33°, according to Tollens' data.[15] It will be noted that the determined rotation is considerably less than this. Furthermore, the determined reducing power is much less than the above simple mixture of sugars should possess. This discrepancy invalidates calculation of the percentages of sugars by simultaneous equations, as given by Browne.[16] It appears, therefore, that compounds other than the sugars identified are present, or the latter are not entirely free, or both conditions may exist. The results are suggestive of glucosidic di- and trisaccharides analogous to those described by Browne.[17] The water-soluble fraction recovered from hydrolysis appears to be a product, or products, of partial hydrolysis.

Preliminary tests indicate that it may be possible to extract a gum-like substance from apple wood which will correspond in composition to the alcohol-soluble fraction of the products of hydrolysis. Somewhat similar polysaccharide materials are mentioned by Tollens[18] and by Czapek.[19]

[14] Neuberg, C., *Ber. chem. Ges.*, 1900, xxxiii, 2248.

[15] Tollens, B., Kurzes Handbuch der Kohlenhydrate, Leipsic, 3rd edition, 1914, 128, 175, 286.

[16] Browne, C. A., A handbook of sugar analysis, New York, 1912, 477, 489.

[17] Browne,[16] pp. 643, 731.

[18] Tollens,[15] pp. 474, 563.

[19] Czapek, F., Biochemie der Pflanzen, Jena, 2nd edition, 1913, i, 654, 685.

Work in progress shows that the acid-hydrolyzable material of apple wood in the spurs varies in a manner which indicates that it may function as an important reserve carbohydrate material. If substantiated, this relation will throw unusual importance upon the so called hemicellulose fraction of wood, the function of which has hitherto been obscure.

We purpose following the composition of this material as it is deposited in the branches and drawn upon for subsequent growth. It will also be desirable to compare it with corresponding material in trees of other species.

SUMMARY.

Analysis of apple wood from fruiting branches shows a high content of the acid-hydrolyzable material commonly designated as hemicellulose. The alcohol-soluble fraction resulting from partial hydrolysis of this material has been found to contain large amounts of xylose and glucose, with a little galactose. It is suggested that this hydrolyzable material forms a reserve source of carbohydrate in the metabolism of the apple tree.

THE ESTIMATION OF BILE ACIDS IN BILE.

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In a recent paper Foster and Hooper (1) have described a rapid method for the estimation of bile acids in dog bile. The method depends on the fact that the two bile acids usually found in dog bile, *viz.* taurocholic and taurocholeic, on hydrolysis with alkali give taurine, which yields its nitrogen quantitatively in 4 minutes when shaken with HNO_2. Taurocholic and taurocholeic acids do not react with HNO_2 before hydrolysis. The proteins in bile are precipitated by hot alcohol which at the same time holds the bile salts in solution. A portion of the filtrate is evaporated to dryness and then hydrolyzed with 8 per cent NaOH for 6 hours. The taurine thus set free can be estimated from its yield of amino nitrogen. Since bile normally contains a small amount of free amino nitrogen, an estimation to allow for this is carried out on a separate portion of evaporated filtrate. Other nitrogenous constituents present in bile do not yield amino nitrogen with the exception of urea. But as the amount of urea present in bile—32 mg. per 100 cc. (2)—is small and since only about 3 per cent of its nitrogen is liberated in 3 minutes, this factor can be neglected.

While this method is applicable to bile acids which on hydrolysis yield only taurine it does not differentiate between these acids and those having glycocoll in the molecule. Since bile from most animals, especially man, contains acids of both the glycocoll and the taurine series, we have concerned ourselves with the problem of devising a method which will differentiate between the two series of bile acids.

Strecker (3) found that hydrolysis of glycocholic acid by means of alkali gave cholic acid and glycocoll and we have confirmed

his observation by quantitative experiment. A solution containing about 500 mg. of twice crystallized glycocholic acid per 25 cc. yielded 0.0141 gm. of nitrogen by the Kjeldahl method and 0.0142 gm. of nitrogen (corrected for the abnormal value given by glycocoll) when hydrolyzed for 6 hours with 8 per cent NaOH and the amino nitrogen was estimated. Glycocoll, like taurine, is not decomposed by heating with alkali in the concentration and time necessary to hydrolyze glycocholic acid. A solution containing 65 mg. of glycocoll per 25 cc. yielded 11.9 mg. of amino nitrogen and the same value was obtained on adding NaOH to a concentration of 8 per cent and heating at 100°C. for 10 hours. Hydrolysis of a mixture of bile acids of the taurine and glycocoll series and subsequent estimation of the amino nitrogen will give a value which is too high since glycocoll yields by Van Slyke's method 103 per cent of the theoretical nitrogen (4). If, however, the total amino nitrogen and the taurine nitrogen are known the usual correction for the high value given by glycocoll can be made.

In a mixture of pure bile acids taurine can be estimated from its sulfur content. This method has been utilized for its estimation in bile. It is seemingly open to objections since protein-free bile is known to contain sulfur compounds other than taurocholic and taurocholeic acids. Hammarsten (5) states that he has found ethereal sulfates in human and shark bile while von Bergmann (6) could not detect it in dog bile. Estimations of total sulfates which were carried out by us on the specimens of bile reported in this paper did not give a value greater than 1 mg. of $BaSO_4$ per 10 cc. of bile, which is within the experimental error of the method. It is probable that small quantities of taurine, cystine, and jecorin occur in bile but their concentrations are not known. Estimations of phosphorus in protein-free bile showed that the phospholipoid nitrogen is less than 0.1 per cent of the nitrogen present as bile acids and since jecorin is probably only a small fraction of the concentration of phospholipoid nitrogen, no error in the estimation of bile acids on the basis of the total sulfur will result by neglecting this factor. The amount of cystine and taurine cannot be large since the free amino nitrogen present in unhydrolyzed bile is only about 5 per cent of the nitrogen present as bile salts. Undoubtedly a part of this free amino

nitrogen results from heating the protein-free bile. To an alcoholic solution containing 200 mg. of twice recrystallized glycocholic acid sufficient NaOH was added to give a neutral reaction and the solution was evaporated in the usual manner. Estimation of the free amino nitrogen gave a value of 0.12 mg. or 2 per cent of the total bile acid nitrogen. This is not due to the splitting of the glycocholic acid by HNO_2 since shaking for 8 minutes gave a practically identical value; *viz.*, 0.14 mg. Other amino-acids besides taurine and glycocoll are probably also found in bile and contribute to the free amino nitrogen. If in bile containing acids of both the taurine and the glycocoll series we assume that the free amino nitrogen is made up of taurine and glycocoll in the ratio in which they occur in bile combined with cholic acid, the resultant error in the estimation of the taurine series of bile acids on the basis of the total sulfur will be less than the error (6 per cent) found by Foster and Hooper on adding known amounts of taurocholic acid to bile. The method employed by us with biles containing either an excess of glycocholic acid or nearly equal quantities of acids of the taurine and glycocoll series is to subtract the free amino nitrogen from the total obtained after hydrolysis, thus neglecting the sulfur present as taurine. If the bile contains predominantly acids of the taurine series the free amino nitrogen is assumed to consist wholly of taurine and a correction for the sulfur is made.

Croftan (7) has pointed out that the protein precipitate may contain a large proportion of the bile acids and Bang (8) states that taurocholic acid may be precipitated by means of protein solutions. To overcome this factor, Foster and Hooper filter off the protein precipitate after bringing the alcoholic solution to the desired volume and then take an aliquot of the filtrate for the estimation of bile acids. This procedure is based on the assumption that taurocholic acid is uniformly distributed throughout the two phases. We have estimated the error which would result if the amount of taurocholic acid adhering to the protein precipitate after several washings with alcohol is neglected. The proteins from 100 cc. of ox bile were precipitated by 10 volumes of alcohol, filtered, and washed a number of times with alcohol. The sulfur content of the precipitate was 2 mg. and the nitrogen 11 mg. Since the sulfur content of mucin is about 13 per cent

of the nitrogen (9), the amount of taurocholic acid in the precipitate from 10 cc. of bile is less than the experimental error of the method.

Our procedure for the estimation of taurine and glycocoll nitrogen combined as bile acids is as follows: To 10 cc. of bile (more may be necessary if the bile is dilute) 8.5 volumes of alcohol are added, the mixture is heated almost to the boiling point, filtered through a dry filter paper, and repeatedly washed with small quantities of alcohol until the volume of the filtrate, when cold, has been brought to 100 cc. Two 20 cc. portions are evaporated to dryness in porcelain dishes and the remainder is evaporated in a nickle crucible. The residue in the crucible is fused with a mixture of Na_2CO_3 and Na_2O_2, the sulfates are precipitated with $BaCl_2$, and weighed as $BaSO_4$. The residue from one of the 20 cc. portions is dissolved in water, brought to a volume of 10 cc., and 2 cc. portions are used for the estimation of the amino nitrogen. The other is washed into a 10 cc. volumetric flask with 8 per cent NaOH, placed in a water bath, and heated at 100°C. for 6 to 8 hours. The amino nitrogen is estimated in 2 cc. of the hydrolysate. The difference between the amino nitrogen in the hydrolyzed and unhydrolyzed bile gives the taurine and glycocoll nitrogen of the bile acids. The taurine nitrogen is calculated from the figures obtained in the estimation of the sulfur. If the bile acids consist predominantly of the taurine series the free amino nitrogen is assumed to consist wholly of taurine and a correction applied to the total sulfur value. The difference between the amino nitrogen due to bile acids and the taurine nitrogen, less 3 per cent, gives the glycocoll nitrogen.

To test the accuracy of this method a known quantity of glycocholic acid, dissolved by addition of NaOH, was added to a specimen of ox bile, Sample B, the bile acids content of which was estimated on a separate portion, Sample A. The following results, per 10 cc. of bile, were obtained:

	Sample A. *mg.*	Sample B. *mg.*
Amino nitrogen after hydrolysis	13.4	15.4
" " before "	0.3	0.4
Nitrogen of the bile acids	13.1	15.0
Taurine nitrogen calculated from the sulfur value (0.0103 S)	4.5	4.5

	Sample A. *mg.*	Sample B. *mg.*
Glycocoll nitrogen (uncorrected)	8.6	10.5
" " (corrected)	8.4	10.2
Nitrogen recovered from bile acid added		1.8
Total nitrogen of the glycocholic acid (66.7 mg.) added		2.0
Free amino nitrogen in bile acid added		0.1
Nitrogen combined as glycocholic acid		1.9
Glycocholic acid recovered, *per cent*		95

The accuracy of the method, based on duplicate estimations, depends to a large extent on the concentration of bile salts. Duplicate sulfur estimations checked within 1 mg. of $BaSO_4$. Since the aliquot (0.6) taken is large the resultant error is usually less than 1 per cent. The chief error lies in the estimation of the amino nitrogen since the aliquot (0.04) taken for analysis is small. Duplicate estimations agree within 3 per cent. Other factors, such as the presence of amino-acids besides taurine and glycocoll, sulfur compounds other than taurine, splitting of bile acids during evaporation of the alcoholic filtrate, etc., probably do not contribute an error greater than 3 per cent. This gives a maximum error of about 6 per cent which probably represents the accuracy of the method.

The analyses reported in Table I were made with the aid of this method. With the exception of the biles from the dog and human fistulas, the specimens were mixed samples obtained from the slaughter house. In ox bile we note that the predominance of acids of the taurine and the glycocoll series, respectively, varies in different specimens. This had been previously noted by Marshall (10) who examined a large number of specimens of ox bile. Pig bile contains chiefly acids of the glycocoll series while bile from the sheep and the dog contains only those of the taurine group. In human bile we note a slight excess of glycocholic acid over the acids of the taurine series. These results are essentially in accord with the analyses reported by other workers using more laborious methods (11).

TABLE I.

Bile.*	Amino N after hydrolysis.	Amino N before hydrolysis.	N in bile acids.	Sulfur.	Taurine N.	Glycocoll N.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Ox 1	0.0182	0.0006	0.0176	0.0162	0.0071	0.0102
" 2	0.0172		0.0172	0.0270	0.0118	0.0052
Pig 1	0.0187	0.0003	0.0184	0.0089	0.0039	0.0141
" 2	0.0202	0.0001	0.0201	0.0076	0.0032	0.0164
" 3	0.0172	0.0008	0.0164	0.0092	0.0040	0.0120
Sheep 1	0.0216	0.0033	0.0183	0.0488	0.0181	None.†
" 2	0.0247	0.0026	0.0221	0.0526	0.0204	" †
Dog 1	0.0023	0.0004	0.0019	0.0057	0.0021	" †
" 2	0.0024	0.0004	0.0020	0.0058	0.0021	" †
Human 1	0.0017	0.0001	0.0016	0.0019	0.0008	0.0008
" 2	0.0013	0.0001	0.0012	0.0013	0.0006	0.0006
" 3	0.0018	0.0003	0.0015	0.0016	0.0007	0.0008‡
" 4	0.0086	0.0009	0.0077	0.0067	0.0029	0.0047§

* All figures are per 10 cc. of bile.

† Calculations are based on the assumption that the free NH_2 is due wholly to taurine.

‡ Slight decomposition.

§ Obtained on autopsy.

SUMMARY.

A method for the estimation of the nitrogen of bile acids of the taurine and the glycocoll series in bile is described. It is based on the fact that on hydrolysis with NaOH the bile acids are split, setting free taurine and glycocoll which react quantitatively with HNO_2. The taurine nitrogen can be estimated from the total sulfur content of protein-free bile. The difference between the amino nitrogen resulting from the splitting of the bile acids, and the taurine nitrogen, less 3 per cent, gives the glycocoll nitrogen.

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THE EFFECT OF ALKALI ON THE EFFICIENCY OF THE WATER-SOLUBLE VITAMINE B.*

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(Received for publication, December 22, 1920.)

While there is convincing evidence that the growth-promoting capacity of the water-soluble vitamine B is little, if at all, affected by heating with relatively strong acids, even for many hours, comparatively little is to be found in the literature respecting the effect of alkalies on this vitamine. Thus McCollum and Simmonds,[1] Drummond,[2] and Voegtlin and Lake[3] agree that heating with more or less dilute alkalies destroys the water-soluble vitamine B, while on the other hand Daniels and McClurg[4] and Whipple[5] state that under the conditions of their experiments it was not destroyed.

Without entering into a critical discussion of these conflicting views until our knowledge of this subject is more complete we wish to record some observations which we have made on a concentrated preparation of this vitamine obtained from brewery yeast according to the method of Osborne and Wakeman.[6]

The preparation used for these experiments sufficed in daily doses of 12 mg. to promote the prompt recovery of young rats which had failed on a diet adequate in respect to every factor

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, Washington, D. C.

[1] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, xxxiii, 55.

[2] Drummond, J. C., *Biochem. J.*, 1916–17, xi, 255.

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except the water-soluble vitamine B (see Chart I, Rats 5917 and 5924).

An aqueous solution of 1 gm. of this preparation required 0.0530 gm. of NaOH to make its reaction neutral to litmus. Accordingly 5 gm. were dissolved in 67.4 cc. of water and 32.6 cc. of a solution of NaOH containing 0.6650 gm. were added. This sufficed to neutralize the acidity of the preparation and leave an excess equivalent to a 0.1 N solution of the alkali. After

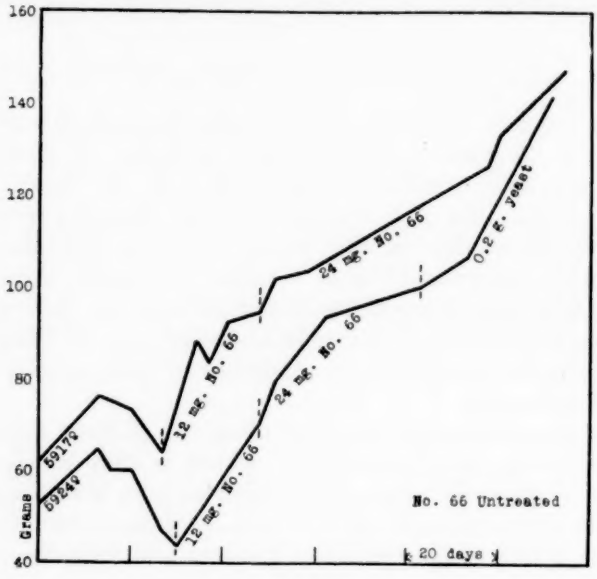

CHART I.

this alkaline solution had stood for ½ hour at a temperature of 20°, 22 cc. of 0.1 N HCl were added to 20 cc., thus making the reaction faintly acid to litmus. After standing over night at 0°, the solution was mixed with 5.67 gm. of starch, evaporated on the steam bath, and made into tablets, each containing 15 mg. of the vitamine preparation, No. 88.

After the main alkaline solution of the vitamine preparation had stood at about 20° for 18 hours, 22 cc. of 0.1 N HCl were added to another portion of 20 cc. and the slightly acid solution was evaporated on starch and made into tablets as above described, No. 90.

A third lot of tablets was made in exactly the same manner after the solution had stood at room temperature for 90 hours, No. 91.

A fourth portion of 20 cc., after standing at room temperature for 18 hours, was heated in a bath of boiling water to 90°, kept

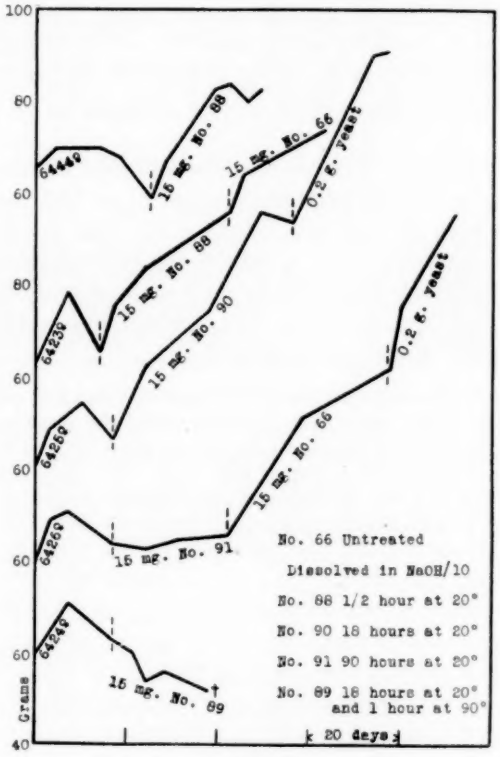

CHART II.

immersed therein for 1 hour longer, 22 cc. of 0.1 N HCl were added, and tablets made as before, No. 89.

Chart I shows the efficiency of the untreated yeast fraction, No. 66, in promoting the recovery and growth of young rats

declining on a diet[7] free from water-soluble vitamine B (Rats 5917 and 5924). That this efficiency was not appreciably impaired by being dissolved in 0.1 N NaOH solution during ½ hour at 20° is shown by Chart II (Rats 6444 and 6423) nor by even 18 hours at 20° (Rat 6425). That, however, it was seriously affected after 90 hours at 20° is shown by the failure of Rat 6426 to recover when supplied with 15 mg. daily of the fraction thus treated, and its prompt gain in weight when the alkali-treated vitamine was replaced by a like quantity of the untreated preparation. That heating in 0.1 N NaOH solution, on the other hand, quickly destroys the activity of this preparation is shown by Rat 6424.

These experiments confirm the observations of those investigators who have reported the destruction of the water-soluble vitamine B when heated with alkalies. It thus appears that in attempting to concentrate, or isolate, the water-soluble vitamine B dilute alkaline solutions can be used without materially affecting its activity provided a low temperature and a short time of exposure to the alkali are employed.

[7] The diet consisted of meat residue 20, salt mixture 4, starch 52, butter fat 9, and lard 15 per cent. The preparation of the meat residue is described by Osborne, Wakeman, and Ferry (Osborne, T. B., Wakeman, A. J., and Ferry, E. L., *J. Biol. Chem.*, 1919, xxxix, 35). The composition of the salt mixture is given by Osborne and Mendel (Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 572).

A METHOD FOR THE DETERMINATION OF CHLORINE IN SOLID TISSUES.

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(Received for publication, December 29, 1920.)

In the course of some investigations in inorganic metabolism it became necessary to determine the total chlorine in small samples of various tissues. While several rapid and satisfactory methods have been devised for blood, milk, and other liquids, these are not applicable to solid tissues. The size of the samples available made necessary the use of a method capable of determining less than 5 mg. There is no difficulty in determining this amount in pure solution with sufficient accuracy by the titrations of Bang (1913) or McLean and Van Slyke (1915), and the problem was simply one of removing the organic matter. For this purpose the wet ashing process of Neumann (1900, 1902–03) seemed the most suitable and we have adapted this method to our needs.

Neumann's method consists in ashing the tissues with a mixture of dilute nitric and sulfuric acids and distilling the hydrochloric acid thus formed into a standard silver nitrate solution. The excess silver is titrated by Volhard's method after removing all traces of nitrous acid. All rubber must be avoided in the apparatus since it is attacked by the hydrochloric acid in the hot gases, causing low results. Plimmer (1904) called attention to the formation of hydrocyanic acid as a source of error in this method and recommended boiling the acid silver solution containing the precipitate for ½ hour before titrating. With this modification, which Neumann (1904–05) later admitted to be necessary, Plimmer secured accurate results on proteins.

To adapt this method to our needs, we have modified the apparatus to deal with small samples of tissue, and have substituted

the more sensitive titration of McLean and Van Slyke for that of Volhard. We have also shortened the time by using a more vigorous digestion mixture.

Neumann used for the digestion a mixture of equal parts of water, concentrated sulfuric acid, and concentrated nitric acid. He found that if more concentrated acid was used, some free chlorine was formed which was not absorbed in the silver solution. We use for our digestion concentrated sulfuric acid and persulfuric acid. This gives extremely rapid digestion but forms a large proportion of free chlorine. Accordingly we do not absorb the gases in the silver solution but in an alkaline sulfite solution. The sulfite is not added as such but is formed in the alkali by absorption of sulfur dioxide from the digestion gases. Plimmer (1904) found that no cyanides were formed by ashing proteins with sulfuric acid alone or in combination with potassium permanganate or manganese dioxide. We have never been able to detect cyanides in our distillates.

Apparatus.

Our apparatus is arranged as shown in Fig. 1. We use suction rather than the pressure of the boiling digestion mixture to carry the gases through the absorption tubes. The tube *A* carries at one end a small bulb *B* perforated with a number of small holes and is provided at the other end with a glass stopper *C*. The tube *A* is passed through the T-tube *D* and attached to it by a piece of rubber tubing. A bent side arm terminating in the bulb *E* is attached just below the glass stopper *C*. A second absorption tube *F* is attached to the side arm of the T-tube *D* and the outlet of this second tube is connected to the suction. The digestion is carried out in the hard glass tube *G*. The internal diameter of *G* is about 1 mm. greater than the external diameter of the bulb *E*. When suction is applied, air enters the annular space between *E* and *G* and carries the gases through the absorption tubes. This space is so narrow and the speed of the entering gases so great, that none of the digestion gases can escape. The apparatus as described presupposes a halogen-free atmosphere. The entering air can be washed, if necessary, by modifying the apparatus as in Fig. 2. *G* and *E* are, as in Fig. 1, the digestion

tube and the bulb. The washed air enters the side arm of the T-tube *H*. The speed of the entering air prevents any gases passing *E* and attacking the rubber stopper. It is necessary to provide a joint in the side arm to allow the replacement of the stopper and rubber tube.

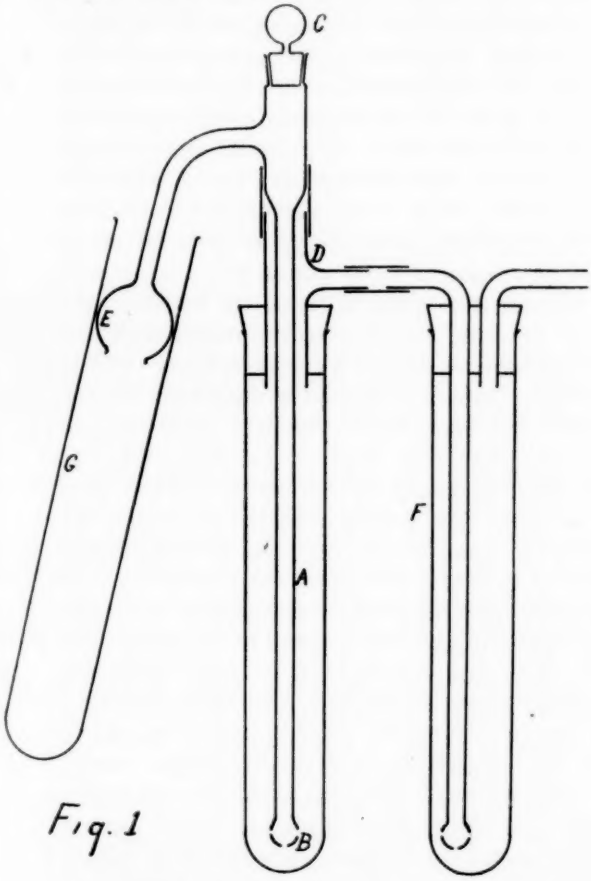

Fig. 1

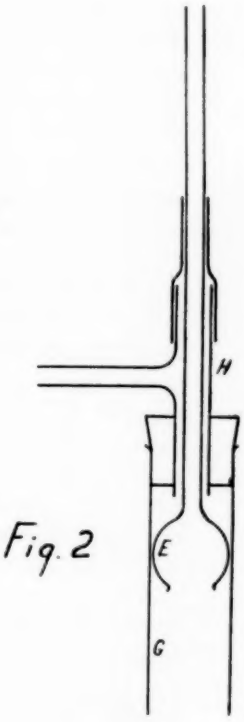

Fig. 2

It can be seen that the gases come in contact with nothing but glass until they have passed through the liquid in the first absorption tube when they are cool and nearly free from hydrochloric acid and chlorine. We have found that only 1 or 2 per cent of the total chlorine escapes absorption in the first tube.

Technique of the Determination.

The actual determination is carried out as follows. The weighed sample of tissue is placed in the digestion tube *G* with a minute drop of mercury as a catalyzer, and two or three pieces of quartz to prevent bumping. (Small pieces of broken silica dishes are much superior to pebbles for this purpose on account of their sharp edges. Broken silica ware may be bought quite cheaply from dealers.) 2 gm. of solid, chlorine-free sodium carbonate (monohydrate) for each gram of tissue sample are placed in the first absorption tube and about 10 cc. of distilled water added. In the second absorption tube are placed about 1 gm. of carbonate and 10 cc. of water. A few drops of caprylic alcohol to prevent foaming are added to each tube and the apparatus is set up as shown. A rather rapid current of air is started and 2 cc. of concentrated sulfuric acid for each gram of tissue are added to the digestion tube above the bulb *E*. The acid is mixed with any liquid in the tube by gentle shaking and the tube is then gently heated until the large pieces of tissue have dissolved. If the heating is too rapid at first, explosions of steam from the interior of the tissue may force gases past the bulb *E*. The tube *G* is inclined so that water condensing on the bulb will not drop directly into the hot acid. The heat is then increased and the acid boiled vigorously for about 5 minutes. The tube is allowed to cool for a minute or two, the bulb is removed, and 2 gm. of ammonium or potassium persulfate and a fresh piece of quartz are added, the bulb being replaced as quickly as possible. The heating is resumed and if, after boiling a few minutes, the acid is not decolorized, another gram of persulfate is added in the same manner. The digestion is continued, adding more persulfate if necessary, until the acid is perfectly clear and colorless.

When the digestion has been completed, the air current is stopped and all the connecting tubes are washed into the two absorption tubes by removing the various joints and the stopper *C*.

The liquid in these tubes is then transferred quantitatively to a 150 cc. Erlenmeyer flask, a few pieces of quartz and a few drops of methyl orange are added, and the mouth of the flask is closed by a bulb having a small hole in its side and a short open stem hanging inside the flask. Dilute (30 per cent) sulfuric acid is

added through the side hole in this trap by means of a pipette until the solution is slightly acid. The solution is boiled for a few minutes, keeping faintly acid to methyl orange by adding a few drops of acid from time to time. An excess of acid is to be avoided as it decreases the sensitiveness of the end-point of the silver titration. When no more sulfur dioxide or carbon dioxide comes off, 5 cc. of standard silver nitrate solution (see below) are added and the solution is evaporated to about 15 cc. The liquid is then transferred to a 25 cc. volumetric flask, cooled, made up to volume, and centrifuged. The silver chloride is well coagulated by the boiling and is easily thrown down in the centrifuge. We prefer to centrifuge rather than filter on account of the difficulty in securing filter paper free from traces of halogens. 20 cc. of the clear liquid are taken for the titration.

Titration.

The solutions used for the titration are those described by Van Slyke and Donleavy (1919) except that the picric acid is omitted from the silver solution. These solutions are made up as follows.

Silver Solution.—5.812 gm. of pure fused silver nitrate, and 250 cc. of nitric acid (specific gravity 1.42) are made up to 1 liter with distilled water.

Potassium Iodide Solution.—2.4 gm. of potassium iodide are dissolved in 1 liter of distilled water, titrated against the silver solution in the manner described by Van Slyke and Donleavy, and diluted so that 12.65 cc. equal 5 cc. of the silver solution.

Indicator Solution.—2.5 gm. of soluble starch are dissolved in about 500 cc. of distilled water, 446 gm. of crystalline sodium citrate ($Na_3C_6H_5O_7.5\frac{1}{2}H_2O$) and 20 gm. of sodium nitrite are added and dissolved by heat. The solution is filtered through cotton, cooled, and made up to 1 liter. It should be noted that according to U. S. P. IX, sodium citrate contains only 2 molecules of water, while according to U. S. P. VIII, it contains the usual $5\frac{1}{2}$ molecules. If the drier preparation is used, proportionately less should be taken. The use of citrate free from chlorides appreciably sharpens the end-point.

The calculation is simple:

$$\frac{1015 - (100 \times \text{cc. KI})}{\text{weight tissue in gm.}} = \text{mg. NaCl per 100 gm. tissue, or}$$

$$\frac{1015 - (100 \times \text{cc. KI})}{1.65\ (\text{weight tissue in gm.})} = \text{mg. Cl per 100 gm. tissue}$$

The color of the methyl orange which is used during the evaporation is entirely destroyed by the nitrous acid formed on adding the indicator solution and does not interfere with the end-point of the titration. The iodide solution should be frequently compared with the silver. If they are found not to agree, the comparison should be repeated using a new indicator solution, since substances are occasionally formed in the indicator on standing which cause the blue color to appear before all the silver has been precipitated. The solutions should be compared whenever a new indicator solution is made up.

Reagents.

All the reagents, including the distilled water, must be frequently tested to insure the absence of halogens, and it is advisable to run blanks often.

Good grades of sulfuric and nitric acids are usually sufficiently free from halogens and we have never had any samples of persulfate which were not pure. Potassium persulfate may be recrystallized easily from water. The ammonium salt is decomposed by hot water but may be converted into the potassium compound by adding a potassium salt to its solution, since the potassium salt is much less soluble.

The purity of the alkali is most important since large amounts of it are used. We have been unable to buy or prepare sodium hydroxide free from halogens. Even when prepared from metallic sodium it contains too much chlorine. However, the preparation of chlorine-free carbonate is quite simple.

400 gm. of $Na_2CO_3.10H_2O$ or an equivalent amount of a drier preparation are dissolved in water and made up to 500 cc. Alcohol is added in small quantities with shaking until the mixture just begins to separate into two layers. About 100 cc. of alcohol are required. The upper alcoholic layer should be as small as possible. The mixture is seeded with about a gram of solid

carbonate and cooled under the tap to below 18°, with constant shaking to prevent the crystals from adhering to the sides of the vessel as they form. About 250 cc. more of alcohol are added and the mixture is again cooled until no more carbonate separates. The crystals are sucked dry on a Buchner funnel, and washed with about 200 cc. of alcohol in small portions. When nearly dry, the carbonate is made up to 500 cc. with distilled water and recrystallized in exactly the same manner as before. This second crystallization and washing should be done with alcohol free from chlorides, which may readily be obtained by distilling over a little carbonate. The purified salt is best preserved as the monohydrate.

If the original preparation is nearly free from chlorides, the first crystallization may be omitted. In all cases the final product should be again tested for chlorides just before using.

If desired, sodium citrate for the indicator solution may be recrystallized in a similar manner.

RESULTS.

Table I gives some results obtained on blood by this method and by Foster's (1917) modification of McLean and Van Slyke's

TABLE I.

NaCl Content of Blood and Plasma, per 100 Cc.

No.	Tissue.	Van Slyke-Foster.	Digestion method.
		mg.	*mg.*
1	Defibrinated blood	491	495
2	Whole blood, normal	494	504
3	" " "	494	506
4	" " "	506	511
5	" " "	438	433
6	" " "	471	468
7	" " "	425	427
8	" " "	475	478
9	Plasma, normal	644	641
10	" "	638	635
11	" "	631	631
12	" "	700	692
13	" "	596	591
14	" "	594	605

method. The original method of McLean and Van Slyke could not be used on account of the lack of Merck's blood charcoal. 1 cc. of blood was used in all cases.

TABLE II.

NaCl Content of Human Rectus Muscle, per 100 Gm.

No.	Diagnosis.	Weight of sample.	NaCl
		gm.	*mg.*
1	Normal	4.067	84
		5.200	82
2	Unknown	3.55	108
		3.98	107
		3.275	107
3	Streptococcus septicemia	3.45	97
		4.508	94
4	Tuberculosis	3.108	169
		2.585	168
5	Pulmonary tuberculosis	4.337	158
		4.378	157
6	Tuberculosis; pneumonia	2.903	124
		3.137	125
7	Diphtheria; empyema	3.855	103
		3.675	106
8	Empyema; surgical shock	4.921	119
		3.407	117
9	Miliary tuberculosis	2.941	149
		2.997	147

We have used this method successfully on many different tissues including various abdominal organs, bone, brain, skin, and muscle. Certain tissues may require slight modifications of the digestion. It is often necessary to add more acid during the digestion of tissues containing much inorganic matter such as bone. Tissues containing much fat are among the most resistant,

and it is best to prolong the digestion with acid alone, before adding the persulfate.

Table II shows the agreement of duplicates obtained on human muscle.

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THE DETERMINATION OF CHLORIDES IN TRICHLOROACETIC ACID FILTRATES FROM WHOLE BLOOD AND PLASMA.

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(Received for publication, December 1, 1920.)

There are at present at least three methods commonly in use for the determination of chlorides in blood. They are the Van Slyke and Donleavy,[1] Foster,[2] and Rappleye[3] methods. The first two are adaptations of the original McLean and Van Slyke[4] starch-iodide titration, merely employing different procedures for the removal of blood proteins, while the latter method utilizes the Volhard titration and another modification for precipitation of blood proteins. It would seem unnecessary to add an additional method to these apparently satisfactory ones but certain considerations seem to the writer to justify the introduction of the present modification of the original McLean-Van Slyke titration.

The method of Foster gives reliable figures and is very satisfactory for a large proportion of blood chloride determinations but frequently there are bloods, especially pathological, from the filtrates of which it is impossible to flock out the silver chloride completely. This happens in spite of large additions of magnesium sulfate and careful fusing of the *m*-phosphoric acid before the precipitation of blood proteins. This is a very serious drawback as a satisfactory remedy (heating) would make the method complicated. Two bloods which presented this difficulty with Foster's method gave no trouble with the method to be described.

While comparing the method of Van Slyke and Donleavy with that of Foster it was found that the latter gave results much lower than the former. This was rather surprising and it was not

[1] Van Slyke, D. D., and Donleavy, J. J., *J. Biol. Chem.*, 1919, xxxvii, 551.

[2] Foster, G. L., *J. Biol. Chem.*, 1917, xxxi, 483.

[3] Rappleye, W. C., *J. Biol. Chem.*, 1918, xxxv, 509.

[4] McLean, F. C., and Van Slyke, D. D., *J. Am. Chem. Soc.*, 1915, xxxvii, 1128.

until the silver solutions for both methods had been again carefully prepared and blanks and known chloride solutions had been determined simultaneously with the bloods that the former figures were considered reliable. Invariably the method of Van Slyke and Donleavy gave correct figures on known solutions of chloride in water and high figures on plasma. These figures were further confirmed by comparing with the method of McLean and Van Slyke[5] and also with the digestion method of Bell and Doisy[6] which through the kindness of Professor Bell the writer was permitted to use.

A probable explanation for the high figures by the method of Van Slyke and Donleavy is that some silver is carried down with the blood proteins during their simultaneous precipitation with the chlorides. It might be mentioned that plasmas used for comparison were carefully obtained to insure freedom from hemolysis although it is doubtful whether a small amount of hemolysis would introduce an appreciable error (see Table I). The method of Rappleye was not compared with that of Bell and Doisy but it would seem to be open to the same error as that of Van Slyke and Donleavy.

This left the problem of finding some blood protein precipitant which would satisfactorily remove blood proteins and give a filtrate from which silver chloride could easily be precipitated. Picric acid with nitric acid used by Austin and Van Slyke[7] for whole blood is open to the same difficulty as *m*-phosphoric acid. The same is also true of nitric acid with magnesium sulfate. Tungstic acid filtrates, even after removing the excess tungstate with barium hydroxide, while being well suited for the precipitation of silver chloride, gave slightly high results (about 2 per cent). Heat with acetic acid as used by McLean and Van Slyke in their original method is rather laborious and necessitates Merck's blood charcoal. Trichloroacetic acid, a very efficient protein precipitant and one introduced by Greenwald[8] for precipitation of blood proteins, was tried and found to be very satisfactory in the determination of blood chlorides.

[5] McLean, F. C., and Van Slyke, D.D., *J. Biol. Chem.*, 1915, xxi, 361.
[6] Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, 1920-21, xlv, 427.
[7] Austin, J. H., and Van Slyke, D. D., *J. Biol. Chem.*, 1920, xli, 345.
[8] Greenwald, I., *J. Biol. Chem.*, 1915, xxi, 61.

TABLE I.

Sodium Chloride per 100 Cc. of Blood.

	Bell and Doisy.	Trichloroacetic acid filtrate.	Van Slyke and Donleavy.	McLean and Van Slyke.	Foster.	von Korányi.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Plasma, normal		612	649	618	618	
" "		608	641	609	615	
" *		607	645	633		
" normal	636	640	665		640	
	641	640			641	
" "			635	606		
" " †	643	646	688			
	647					
" "	640	645	676			
	645	646				
" diabetic‡	618	628	649			
		628				
" normal	631		661			
	632		662			
" "	629		665			
	630					
" "	609		638			
	611					
" nephritic	595	608				
	609	608				
" normal (diluted)	460	465				
	465	466				
" diabetic	601	607				
	607	608				
Whole blood, normal	466	460				
		462				
Plasma, same blood		629				
		630				
Whole blood, diabetic	487	483				
	491	484				
" "	499	500				
	502	501				
Ascitic fluid		679				676
Plasma, nephritic		588				583
Ascitic fluid, nephritic		623				625

* Mixture of diabetic plasmas. Hemolyzed. Considerable lipemia.

† Very slightly hemolyzed.

‡ Considerably hemolyzed. Plasma had stood 24 hours at 27°C. in an open tube.

Trichloroacetic acid would on first thought seem to be a rather dangerous reagent to use in connection with the determination of blood chlorides since it contains chlorine. Moreover this chlorine will react with silver nitrate in solutions of trichloroacetic acid which have stood for some time. A fresh solution will give no opalescence with silver nitrate, providing it is prepared from chloride-free crystals. At the end of 24 hours, if silver nitrate is added, a slight opalescence will appear but not enough to make an error outside the limit of error of the method. The fact that the solution of trichloroacetic acid even at the end of 24 hours will introduce no appreciable error into the results gives a wide margin of time for a determination.[9]

Trichloroacetic acid filtrates are adapted for the determination of chlorides in plasma, whole blood, or other body fluids. Moreover, the trichloroacetic acid solution is very easily made up and requires no previous preparation as does *m*-phosphoric acid. The filtrates are identical with those in which Bell and Doisy[10] determine phosphates and are also adapted for the determination of non-protein nitrogen according to Folin and Wu[11] (the nitrogen values are slightly higher than in tungstic acid filtrates) so that these determinations may be done simultaneously with the chlorides.

While the starch-iodide titration is superior to the Volhard titration for chlorides in blood, one may get into serious difficulty by not understanding thoroughly the various factors which deter-

[9] A 25 per cent solution of trichloroacetic acid, containing 10 cc. of concentrated nitric acid per 100 cc., which had stood for 2 weeks exposed to ordinary daylight and which gave a strong opalescence with silver nitrate, when used on a solution of sodium chloride in water gave a result only 1.5 per cent too high.

A 20 per cent solution of trichloroacetic acid, which had stood in ordinary daylight for 19 days, when used on a plasma and compared with a freshly prepared solution of trichloroacetic acid on the same plasma gave 632 and 631 mg. of NaCl as compared with 620 and 623 mg. of NaCl for the fresh solution.

A trichloroacetic acid blood filtrate which was determined immediately and after standing 5 days gave the values 609 and 608 respectively, the slightly lower figure on standing being within the limit of error of the titration.

[10] Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, 1920, xliv, 55.

[11] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81.

mine its sensitiveness. As McLean and Van Slyke have shown in their first paper, the final acidity is the most important factor. The blue color is developed best in slightly acid solution. Too much acid abolishes the blue color and leaves only the brown color of the iodine liberated by the nitrous acid. If the solution is not acid enough no color at all will be formed because it is necessary for nitrous acid to be present to liberate hydrogen iodide from the potassium iodide. (This is a valuable criterion to use in determining roughly whether the proper amount of citrate solution has been added, for when a blue color is formed after the addition of each drop of potassium iodide during the titration it is an indication that the optimum conditions are approximated, although this should be checked up with an end-point determination as will be described.)

While using Foster's method it was noticed that the end-point was much sharper and bluer in color than for the other methods. Upon investigation of the cause it was found that the *m*-phosphoric acid present seemed to increase the sensitiveness of the end-point. Further investigation showed that *o*-phosphoric acid or its sodium or potassium salts have the same effect. This is not entirely due to the buffer action of the phosphate for in a titration which was too acid (with nitric acid), due to too little citrate solution having been added, the addition of a small amount of phosphoric acid, notwithstanding that this made the solution more acid, improved the end-point. The addition of phosphoric acid to the citrate solution also greatly increases its flexibility. A variation greater than 0.25 cc. of the McLean-Van Slyke citrate solution will appreciably affect the end-point while a similar variation of the same solution containing a proper amount of phosphoric acid will hardly be noticeable. The most curious fact about the addition of phosphoric acid is that it will make the color of the end-point a deeper blue, even though the optimum amount of citrate solution has been added.

Another important factor influencing the sensitiveness of the end-point is the amount and character of the starch used. Increasing the amount of starch in a titration makes the end-point more intense and therefore easier to distinguish. The starch should be soluble but this can be carried too far as has evidently been done in the soluble starches obtainable on the market at

present. They give an end-point which is not a pure blue, indicating that some dextrin is present. This difficulty may be overcome by boiling ordinary corn-starch 4 or 5 hours and then filtering through cotton. This gives a soluble starch which shows an intense blue color in the presence of iodine. The writer feels that it is more satisfactory to keep the starch solution separate from the citrate solution.

The presence of other salts during the titration makes the end-point less sharp. Too much nitrate, magnesium sulfate, or sodium chloride are examples. While it is not imperative it is very desirable for this reason to use sodium citrate, sodium nitrite, and phosphoric acid free from chlorides.

The final volume for titration bears an inverse ratio to the intensity and sharpness of the end-point. This makes it desirable to perform the titration in as small a volume as possible.

Finally as pointed out by Van Slyke and Donleavy the blue color is not produced by the starch until a considerable but definite excess of potassium iodide solution has been added. They give this amount as 0.15 cc. but it may vary, even under optimum conditions, with the character of the starch and other factors discussed above. It is therefore very important to determine the amount of this excess for every new buffer solution, silver nitrate solution, or starch solution.

Bearing in mind the above factors it has been the purpose of the writer to make the titration as sensitive and flexible as possible. The final volume for titration has been considerably reduced, the starch solution has been made from corn-starch and kept separate, phosphoric acid has been added to the citrate solution, and finally the potassium iodide solution has been made slightly stronger.

Preparation of Solutions.

1. Trichloroacetic Acid 20 Per Cent.—A simple procedure for making this solution when needed is as follows: Counterpoise a small beaker or flask on ordinary laboratory scales. Weigh directly into the beaker or flask enough trichloroacetic acid crystals to give a 20 per cent solution of the volume desired. Then add the proper amount of distilled water with a pipette or small cylinder. Great accuracy is not required in making up this solution.

2. Starch Solution.—Place 5 gm. of corn-starch or potato starch in a liter beaker containing about 400 cc. of distilled water. Heat the mixture to

boiling with stirring to prevent scorching. After it once begins to boil the stirring is no longer necessary. Continue the boiling gently for 5 hours keeping the volume of the fluid at about 400 cc. by adding distilled water from time to time. Then pour the solution into a liter flask and make to 1,000 cc. when cool. Filter through a good thickness of cotton and the solution is then ready for use. The solution is quite opalescent but gradually settles out forming a clear supernatant portion. It is not necessary to wait for this before using. (The solution can be cleared by filtering through filter paper but it soon fills the pores and the filtration is extremely slow.) Add about 10 drops of xylene or toluene and thoroughly mix with the solution. This will prevent the formation of moulds or growth of bacteria. Such a solution prepared by the writer has kept perfectly for 5 months with contant use.[12]

3. Buffer Solution.—Place in a liter flask 446 gm. of sodium citrate ($5\frac{1}{2}$ mols H_2O) and 50 cc. of 80 per cent phosphoric acid syrup. Make the whole up to a volume of about 800 cc. with distilled water and dissolve with the aid of boiling. Pour this solution quantitatively into a liter volumetric flask and allow to cool. To about 100 cc. of distilled water add 20 gm. of sodium nitrite. This will dissolve without the aid of heat. When it is dissolved, pour into the cooled citrate-phosphate solution, mix, and make to volume. This solution may then be filtered, if necessary, and it is ready for use.[12]

4. Silver Nitrate Solution.—

Silver nitrate	11.624 gm.
Nitric acid (sp. gr. 1.42)	390 cc.

Make to 1,000 cc. with distilled water.

1 cc. of this solution is equivalent to 4 mg. of NaCl.

5. Potassium Iodide Solution.—1 cc. of this solution is equivalent to 0.8333 mg. of NaCl.

Dissolve 6.0 gm. of potassium iodide in 2,000 cc. of distilled water. Standardize this solution against the silver nitrate solution as follows: To 10 cc. of distilled water, accurately measured, add 2 cc. of silver nitrate solution from an Ostwald pipette accurately standardized for blow-out delivery. After mixing, pipette 10 cc. of this solution into a 50 cc. Erlenmeyer flask and then add 2.5 cc. of the buffer solution and 1.0 cc. of the starch solution. (Mohr pipettes may be used for the buffer and starch solutions.) Titrate with the potassium iodide solution to the first permanent blue color. It should take about 8.02 to 8.06 cc. as discussed under "End-point." Make the necessary dilution of the potassium iodide solution and then carefully standardize again both for end-point (see "End-point") and strength.

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[12] This solution may be used in the titration of either Foster's method or the method of Bell and Doisy by using double the quantity required for the titration described in this paper.

End-Point.

The end-point should be a pure, permanent blue. The permanency of the end-point may be determined by shaking gently for 30 seconds to see if it fades. I have found it desirable to run the titration not to the first permanent blue but 1 drop past this point to a deeper blue. If the potassium iodide solution is standardized for such an end-point the procedure is unquestionable. All subsequent titrations should be run to the same depth of color. When about 0.2 cc. from the end-point, if 5 or 6 drops of potassium iodide solution are added at once a false end-point will appear and fade in about 15 to 45 seconds. This is due to the presence of chlorides in the indicator solutions. It is therefore best to approach the end-point a drop at a time. This will insure a definite and permanent end-point upon the addition of 1 drop (0.02 cc.) of potassium iodide and no confusion will result from fading and necessary addition of more potassium iodide until the permanent color is obtained.

The optimum conditions for the end-point for any particular set of solutions used may easily be determined. Place 5 cc. of a solution containing 850 to 900 mg. of NaCl per 100 cc. in a 50 cc. volumetric flask, add 5 cc. of 20 per cent trichloroacetic acid, make to the mark with distilled water, and mix. Accurately measure four 10 cc. portions into four 15 cc. centrifuge tubes. Add 2 cc. of the silver nitrate solution to each tube with an Ostwald pipette, insert a clean, dry stopper, vigorously shake the tube for a moment to flock out the silver chloride, and centrifuge. Accurately pipette 10 cc. of the supernatant fluid in each tube into 50 cc. Erlenmeyer flasks. Add 2.0, 2.5, 3.0, and 3.5 cc. portions of the buffer solution respectively to the four flasks. Then add 1.0 cc. of the starch solution to each flask and titrate[13] each to the desired permanent blue color. As all the silver nitrate will have been removed by the sodium chloride, the amount of potassium iodide solution added will represent the amount necessary to add

[13] It is best to use a small (5 cc.) burette for the titration as it permits of greater accuracy. The Folin sugar burette (supplied by Emil Greiner Company, New York) is very well adapted as it is of 5 cc. capacity and graduated in 0.02 cc. The tip may be drawn out slightly in the flame to make the drops smaller. It is possible to do this so that 1 drop will be 0.02 cc.

in excess of the neutral point in order to obtain the blue color. Ordinarily the flask containing only 2 cc. of buffer solution will require 0.06 to 0.08 cc. The others will require 0.02 to 0.05 cc. The best color will usually be given by the flask containing 2.5 cc. of buffer solution, and I have found that the end-point is quite intense when 0.04 cc. of the potassium iodide solution has been added. However, as pointed out this will vary with the solutions. When this procedure has been completed the potassium iodide solution can then be accurately diluted so that $8.0x$ cc. will be required for a blank determination where x cc. is the amount required for an optimum end-point.

Procedure for Blood Chloride Determination.

1. Preparation of Blood Filtrate.—Carefully measure (an automatic burette is convenient) 40 cc. of distilled water[14] into a clean, dry flask or tube. Then run 5 cc. of plasma, whole blood, or other fluid to be determined into the water from a pipette graduated to contain 5 cc. (The determination may be done with as little as 2 cc. of plasma with proportional quantities of water and trichloroacetic acid.) Blow out the last drop and then rinse out the pipette twice by sucking up the mixture of water and blood, and finally blowing out the pipette. Accurately add 5 cc. of the trichloroacetic solution, stopper, and shake at intervals for 10 to 15 minutes. Filter the mixture through a dry chloride-free filter[15] into a dry tube or flask. The filtrate is then ready for the chloride or other determinations.

[14] It is impossible to obtain C.P. trichloroacetic acid on the market at present. The U.S.P. grades give a filtrate from which silver chloride will not completely precipitate. This trouble is not experienced with pure trichloroacetic acid. The difficulty is easily remedied by the use of 5 cc. of 95 per cent ethyl alcohol (or ethyl alcohol denatured with methyl alcohol; methyl alcohol alone is not so satisfactory) in the precipitating mixture. This may best be done by adding 100 cc. of alcohol to 700 cc. of distilled water and using 40 cc. of this mixture for diluting the blood. The filtrates are suitable for the other determinations as well as chloride.

[15] The use of chloride-free filter paper should be emphasized. Two chloride determinations on a blood were made using the method of Van Slyke and Donleavy with Alpha paper (chloride-free) and a good grade of Whatman paper. The filtrate from the Whatman paper gave a result 1.2 per cent higher than that from the Alpha paper. Of course this danger is perhaps not so great when solutions containing no silver nitrate are filtered.

2. Chloride Determination.[16]—Measure 10 cc. of the filtrate into a 15 cc. centrifuge tube, add 2 cc. of the silver nitrate solution as has been described, stopper, shake vigorously, and then centrifuge. (The silver chloride, especially in whole blood determinations, will be in very fine suspension appearing to be colloidal but after vigorous shaking and centrifuging a perfectly clear supernatant solution is obtained.) Pipette 10 cc. of the clear supernatant solution into a 50 cc. Erlenmeyer flask and add the proper amount of buffer solution (as indicated by the end-point determination) and 1.0 cc. of starch solution. Titrate to the desired permanent blue color with the potassium iodide solution.[13]

3. Calculation.— $8.0x$ − cc. KI = gm. of NaCl per liter of blood, where x represents the cc. of potassium iodide solution necessary to give the end-point.

It is evident that the highest blood chloride that may be determined is 800 mg. of NaCl per 100 cc. of blood. In a large number of determinations on pathological bloods the writer has never encountered such a high blood chloride. If such a blood should occur, however, the trichloroacetic acid filtrate may be diluted with an equal volume of distilled water and the determination made in the way described. The result is then multiplied by two.

RESULTS.

Table I gives a comparison of figures obtained by the method described with those of Bell and Doisy, Van Slyke and Donleavy, McLean and Van Slyke, and the digestion method of von Korányi.[17] All plasmas, unless otherwise stated, which were used for comparison with the method of Van Slyke and Donleavy were perfectly free from hemolysis. Merck's blood charcoal was used in the McLean-Van Slyke determinations. With each determination a known chloride solution as well as a blank was determined. These

[16] This determination may also be made by placing 10 cc. of filtrate in a 25 cc. flask, adding 5 cc. of a silver nitrate solution containing per liter 5.812 gm. of $AgNO_3$ and 250 cc. of HNO_3 (sp. gr. 1.42), making to mark, centrifuging, and titrating 20 cc. of the clear supernatant solution with N/73.1 KI after the addition of 5 cc. of buffer solution and 2 cc. of starch solution. Calculation: 10. xx − cc. KI = gm. of NaCl per liter of blood, where xx is the amount necessary for the development of the end-point.

[17] von Korányi, A., *Z. klin. Med.*, 1897, xxxiii, 1.

figures are not included in the table but in all cases the variation from theoretical was less than 1 per cent.

Table II gives a series of nine complete determinations on the same plasma in comparison with the digestion method of von Korányi. The purpose of this table is to show approximately the

TABLE II.

Comparison of Nine Complete Analyses of a Plasma with That of a von Korányi Digestion Determination.

Trichloroacetic acid filtrates.	von Korányi. 10 cc. plasma used.
mg.	*mg.*
582	583
579	
584	
583	
582	
583	
584	
583	
585	
Average..... 583	

degree of accuracy of the method. With careful work errors should be less than 1 per cent. The titration is capable of even greater accuracy as the variations very probably occur in the precipitation of the blood proteins.

CONCLUSION.

1. The determination of blood chlorides by several different methods is discussed.

2. It is pointed out that the simultaneous precipitation of blood proteins and chlorides is open to error.

3. A modification of the McLean-Van Slyke starch-citrate-nitrite solution is introduced.

4. The use of trichloroacetic acid filtrates for determination of chlorides is described.

I wish to thank Professor Otto Folin and Professor Richard D. Bell for helpful suggestions and encouragement during my work. I am also indebted to Mr. J. C. Whitehorn who kindly made the chloride determinations according to the method of von Korányi.

A SYSTEM OF BLOOD ANALYSIS.*

SUPPLEMENT II.

SIMPLIFIED METHOD FOR THE DETERMINATION OF CHLORIDES IN BLOOD OR PLASMA.

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The method of preparing protein-free filtrates by the use of tungstic acid, as developed and used by Folin, has proved so highly satisfactory in the determination of a number of blood constituents, that it has seemed desirable to apply it to the determination of chlorides.

The procedure developed for this purpose is based upon the principle of the Volhard method;[1] namely, the precipitation of silver chloride from a known amount of silver nitrate and titration of the excess silver nitrate by means of sulfocyanate, using ferric ammonium alum as an indicator.

A number of preliminary experiments were conducted to ascertain the most convenient conditions which would give a good end-point. Efforts to obtain increased accuracy by greatly increased dilution of reagents were disappointing. In order to insure a sharp end-point, the volume of fluid at the end of titration must be kept small, which cannot be done when very dilute solutions are used. As a result of dilution the end-point tends to be yellowish, rather than red, and cannot be accurately perceived, at least by the writer's eye, except in strong daylight. This difficulty in the use of very dilute solutions is somewhat increased by the presence of oxalate, because of the lemon-yellow color of

* By agreement between Mr. Whitehorn and myself, this paper is published as Supplement II of the "System of blood analysis" devised by Folin and Wu.—*Professor Otto Folin.*

[1] Volhard, J., Ueber eine neue methode der maassanalytischen Bestimmung des Silbers, *J. prakt. Chem.*, 1874, ix, 217.

iron oxalate. It was presumably for this reason that Rappleye's method for plasma chlorides[2] required the use of citrated rather than oxalated plasma. The personal element in the reading of such faint end-points is perhaps rather large, for Myers and Short have very recently published fairly accurate results obtained on picric acid filtrates by a modified Volhard-Arnold method in which they used ammonium thiocyanate of only M/117 strength.[3] The writer, however, has found it more satisfactory to use a thiocyanate solution of M/35.5 strength, of which 0.03 cc. gives, under the conditions specified, an unmistakable end-point.

For reasons which will appear below, it was necessary to avoid the customary procedure of filtration or centrifugalization of the silver precipitate. This simplification has been accomplished without loss of accuracy by the liberal use of nitric acid and ferric alum. Accurate results were obtained on known chloride solutions, with or without the addition of small amounts of tungstic acid.

The following method was thereupon developed.

I. Preparation of Protein-Free Filtrates.—The filtrate is prepared by the use of the same reagents as have been described in detail by Folin and Wu[4] for the determination of non-protein nitrogen, urea, uric acid, creatinine, creatine, and sugar. Because even slight variations in the chlorides are significant, great accuracy is necessary. The writer customarily uses volumetric flasks in order to insure an accurate 1:10 dilution. The method is applicable, without alteration, to either whole blood or plasma. An amount of filtrate equivalent to 1 cc. of blood or plasma is needed. Less may be used but with proportionate loss of accuracy.

II. Determination of Chloride Content of Filtrate.—*(a) Reagents Required.*—1. Silver nitrate solution (M/35.46).

2. Potassium (or ammonium) sulfocyanate (M/35.46).

3. Powdered ferric ammonium sulfate ($FeNH_4(SO_4)_2$).

4. Concentrated nitric acid (HNO_3 of specific gravity 1.42).

[2] Rappleye, W. C., A simple application of the Volhard principle for blood plasma chlorides, *J. Biol. Chem.*, 1918, xxxv, 509.

[3] Myers, V. C., and Short, J. J., The estimation of chlorides in blood, *J. Biol. Chem.*, 1920, xliv, 47.

[4] Folin, O., and Wu, H., A system of blood analysis, *J. Biol. Chem.*, 1919, xxxviii, 81.

(*b*) *Procedure.*—Pipette 10 cc. of the protein-free filtrate into a porcelain dish. Add with a pipette 5 cc. of the standard silver nitrate solution and stir thoroughly. Add about 5 cc. of concentrated nitric acid, mix, and let stand for 5 minutes, to permit the flocking out of the silver chloride. Then add with a spatula an abundant amount of ferric ammonium sulfate (about 0.3 gm.) and titrate the excess of silver nitrate with the standard sulfocyanate solution until the definite salmon-red (*not yellow*) color of the ferric sulfocyanate persists in spite of stirring for at least 15 seconds.

(*c*) *Calculation.*—

5.00 − titer (in cc.) = mg. of Cl per cc. of blood (or plasma)

Since each cc. of thiocyanate solution used is equivalent to 1 cc. of silver nitrate solution, the difference between the volume of silver nitrate solution taken and the excess determined by the titration, that is 5−titer, represents the volume which reacted with chloride at the ratio of 1 cc. to 1 mg. of Cl. And the 10 cc. of filtrate taken represents 1 cc. of blood (or plasma).

To convert Cl figures into NaCl figures divide by 0.606. The same result may be more easily obtained by the following rule: To obtain mg. NaCl per 100 cc., divide mg. Cl per liter by 6, and subtract 0.001 of the result. Conversely, to obtain mg. Cl per liter, add to mg. NaCl per 100 cc. 0.001 of itself and multiply by 6.

The examples in Table I illustrate the principles involved in the calculation.

TABLE I.

Specimen.	Titer.	Cl per cc.	NaCl per 100 cc.	
			By simple rule.	By long calculation
	cc.	*mg.*	*mg.*	*mg.*
Filtrate A.....	0.75	(5 − 0.75) = 4.25	$\frac{1}{6}$(4,250) − 7 = 701	701
" B.....	2.25	(5 − 2.25) = 2.75	$\frac{1}{6}$(2,750) − 5 = 453	453
" C.....	1.35	(5 − 1.35) = 3.65	$\frac{1}{6}$(3,650) − 6 = 602	602

(*d*) *Preparation of Reagents.*—Dissolve 4.791 gm. of C.P. silver nitrate in distilled water. Transfer this solution to a liter volumetric flask and make up to the mark with distilled water. Mix

thoroughly and preserve in a brown bottle. 1 cc. = 1 mg. Cl. (It is to be noted that the silver nitrate and nitric acid are not added to the protein-free filtrate simultaneously. To do so may result in the mechanical enclosure of silver nitrate solution within the curds, and a consequent error in the positive direction.)

Because sulfocyanates are hygroscopic, the standard solution should be prepared volumetrically. As an approximation about 3 gm. of KCNS or 2.5 gm. of NH_4CNS should be dissolved in a liter of water. By titration under the conditions specified under "Procedure" and by proper dilution prepare a standard such that 5 cc. are equivalent to 5 cc. of the silver nitrate solution.

The solid ferric alum is used rather than a solution, in order to insure a very high concentration in the mixture to be titrated. It is powdered in order to facilitate its solution.

Remarks.

1. Essentially the same procedure and reagents may be used in determining urine chlorides, except for the silver nitrate solution, which should be of M/7.092 strength. When 5 cc. of urine and 5 cc. of this strong silver solution are used in urine chloride determination the calculation becomes $5 - \frac{\text{titer}}{5} = \text{mg. Cl per cc. of urine.}$

2. When a determination of both urea and chlorides is desired on a small sample, as may sometimes occur in cases where nephritis is suspected, one may pipette 2 cc. of the plasma into a 25 cc. flask, dilute to about 20 cc. with water, add 2 cc. each of the tungstate and acid solutions, make up to the mark with water, and shake. This will give sufficient filtrate for both determinations, 5 cc. for urea by Folin's distillation method and 10 cc. for the chlorides by the method described. In either case the figure obtained must be multiplied by $\frac{5}{4}$, since the filtrate has been diluted 2 : 25 instead of 2 : 20.

3. The glassware should be checked to within at least 0.5 per cent.

4. Reagents must be halogen-free. Some samples of nitric acid contain much chloride. None of the samples of tungstate tested has contained chloride. To guard against the possibility

of contamination, however, all samples should be tested as follows before using: Mix one volume of 10 per cent sodium tungstate solution with two volumes of concentrated, chloride-free nitric acid, and filter into a test-tube containing silver nitrate solution. Turbidity indicates contamination with halogen.

5. At Dr. Folin's suggestion, purification of tungstates containing added chlorides has been accomplished by recrystallization with alcohol. Sodium tungstate containing 0.3 per cent NaCl was so nearly purified by one recrystallization that the above mentioned silver test gave only a faint opalescence, scarcely perceptible even by transmitted light—not enough to produce an appreciable error. After a second recrystallization no chloride whatever could be detected. The following procedure was used: To a cooled 50 per cent solution of the contaminated tungstate, prepared with the aid of heat, add slowly an equal volume of 95 per cent ethyl alcohol and let stand for 10 minutes. Pour the suspension of crystals on a Buchner funnel, wash twice with alcohol, and dry.

6. The writer's attention has very recently been called to the method published by Rieger for chloride estimation on tungstic acid filtrates.[5] Certain features of this article deserve comment.

(*a*) The method therein described retains the centrifugalization or filtration procedure, which at times causes erroneously high results on tungstic acid filtrates, whether or not there is sufficient tungstate present to give a precipitate on the addition of an equal volume of concentrated nitric acid. Incidentally, the absence of such a precipitate does not indicate the absence of tungstate, as Rieger has stated, for solutions containing as much as 25 mg. of sodium tungstate per 100 cc. may not give a precipitate with nitric acid except when heated.

(*b*) The article mentioned contains a method for the "purification of sodium tungstate;" *i.e.*, for the preparation of chloride-free tungstate. It is very difficult to believe that the method so designated really accomplishes its purpose, since it calls for the use of 7 cc. of 40 per cent sodium hydroxide solution for each 10 gm. of sodium tungstate taken. Preparations of sodium hydroxide always contain large amounts of chloride. On the other

[5] Rieger, J. B., The estimation of chlorides in whole blood, *J. Lab. and Clin. Med.*, 1920-21, vi, 44.

hand, none of the samples of sodium tungstate tested by the writer has contained perceptible amounts of chloride. It has therefore not been necessary to purify. Certainly "purification" should not be attempted by the use of sodium hydroxide.

(*c*) Instead of precipitating the plasma proteins before dilution it is preferable to dilute the plasma with distilled water before adding the acid, in order to insure an even distribution of chlorides between solution and precipitate.

(*d*) It is not necessary to wait an hour for protein precipitation. 5 minutes are sufficient. Time of standing before filtering off the precipitated protein causes no appreciable difference in the chloride determination, as shown by the following results on the same plasma: Stood for 5 minutes before filtering, 3.64 mg. Cl per cc.; 15 minutes, 3.68; 30 minutes, 3.68; 1 hour, 3.64; and $3\frac{1}{4}$ hours, 3.70.

Soundness of the Method.

The soundness of the method of chloride determination described obviously depends upon the answers to two questions:

I. Does the titration figure indicate accurately the excess of silver nitrate?

II. Does the amount of silver precipitation so found indicate accurately the chlorides of the plasma?

I. The first of these questions deserves careful attention. Many chemists will quite properly be very skeptical on this point. Sutton states[6] "In cases where chlorine is precipitated by excess of silver, and the excess has to be found by thiocyanate, experience has proved that it is absolutely necessary to filter off the chloride and titrate the filtrate and washings." Rosanoff and Hill[7] have shown that the error is due to the reaction of silver chloride with sulfocyanate. Their figures would indicate that silver chloride, when shaken in an equimolar water solution of ammonium sulfocyanate, reacts so rapidly as to precipitate 43 per cent of the sulfocyanate in 2 minutes. This occurs because

[6] Sutton, F., Systematic handbook of volumetric analysis, Philadelphia, 10th edition, 1911, 145.

[7] Rosanoff, M. A., and Hill, A. E., A necessary modification of Volhard's method for the determination of chlorides, *J. Am. Chem. Soc.*, 1907, xxix, 269.

silver chloride is more soluble than silver sulfocyanate. Harvey[8] has shown, however, that both ferric alum and nitric acid retard this reaction. By using nitric acid in a concentration of 5 per cent, he obtained practically identical results on known chloride solutions and on urines whether or not he filtered off the silver chloride. His results, indeed, indicate a slightly greater accuracy without filtration, but the difference is so small as to be entirely without significance (*e.g.* 0.7052 without filtration and 0.7069 per cent NaCl with filtration on a known 0.7039 per cent NaCl solution).

The following experiments were designed and carried out to test the accuracy of the titration in the presence of silver chloride. Since the purpose was to discover if the presence of silver chloride influenced the accuracy of the titration, all factors such as tungstate which might modify or conceal such an influence, had to be excluded. Therefore a pure solution of sodium chloride was substituted for the blood filtrate. A 0.1 N solution was prepared by dissolving in distilled water 0.5846 gm. of recrystallized, thoroughly dried sodium chloride, and making up to 100 cc. in an accurately calibrated flask. Then 1 cc. of this solution and 9 cc. of water were used instead of 10 cc. of blood filtrate, but all the subsequent details were followed as specified under the heading "Procedure." Eleven determinations were made in this manner. The chlorine content of 1 cc. of 0.1 M NaCl solution, as determined by these experiments, was: 3.55; 3.52; 3.56; 3.56; 3.54; 3.53; 3.56; 3.55; 3.56; 3.55; and 3.54 mg. The average was 3.547, compared to a theoretical value of 3.546 mg. The highest and lowest deviations were 0.7 and −0.3 per cent. The presence of silver chloride during the titration had therefore produced no error.

Similarly the chlorine content of 4 cc. of 0.1 M NaCl solution, as determined in the same manner (which of course necessitated the use of 3 cc. of M/7.092 $AgNO_3$ solution instead of 5 cc. of M/35.46) in three such experiments, was found to be 14.28, 14.23, and 14.30 mg., compared to the theoretical value of 14.2 mg. Here also the presence of chloride produced no error in titration.

As a final crucial test, another determination was made on 1 cc.

[8] Harvey, S. C., The quantitative determination of the chlorids in the urine, *Arch. Int. Med.*, 1910, vi, 12.

of 0.1 M NaCl solution, but with this difference, that the mixture containing silver chloride was centrifugalized and an aliquot portion of the water-clear supernatant liquid taken for titration. The whole mixture measured 15 cc. and the titration of 10 cc. of the supernatant liquid required 0.97 cc. of M/35.46 KCNS solution. Since $5 - \frac{3}{2}(0.97) = 3.55$, it is evident that the method of titration gives the same result whether or not the silver chloride is removed.

The essential part of the procedure is the use of nitric acid, and the principle is a physical one—the flocking out of the silver chloride, with the consequent reduction of the surface exposed for reaction with the sulfocyanate. It is for this purpose that so large an amount of nitric acid is used, approximately 25 per cent of the volume of the mixture in which the titration is carried out.

Much higher concentrations of nitric acid should not be used, as the sulfocyanate is decomposed by them quite rapidly. To determine if such decomposition played an important part in the disappearance of the end-point, a small drop (0.03 cc.) of the M/35.46 KCNS solution was added to each of two dishes, one containing 10 cc. of 30 per cent nitric acid, and the other 10 cc. of 15 per cent nitric acid, and each containing about 0.3 gm. of ferric alum. The red color persisted in the first mixture for 15 minutes, in the second it was still present at the end of 15 hours. It is evident, therefore, that under the conditions which may occur in using this method, the decomposition of sulfocyanate by nitric acid is not rapid enough to affect the accuracy of the titration.

In accordance with the geometrical principle that volumes vary as the cubes of a dimension, whereas surfaces vary as the squares, the surface of the silver chloride, when flocked out, is so small that the reaction with ferric sulfocyanate, although probably still going on, is negligible. (The color due to 0.03 cc. of the M/35.46 KCNS solution persisted for 19 hours in the presence of such curds of silver chloride.) But the small amount of silver chloride which in spite of the nitric acid remains in fine suspension, has a larger aggregate surface and therefore reacts much more rapidly with the sulfocyanate.

Herein lies the explanation of the phenomena observed when KCNS is added to the mixture containing silver nitrate, silver

chloride, ferric alum, and nitric acid. The red color which appears with each drop disappears in about 3 seconds, because the ferric sulfocyanate reacts with the solution of $AgNO_3$. But when all the silver present as $AgNO_3$ has been precipitated, the red color which appears when the next drop is added persists about 30 seconds (from 10 seconds to 1 minute, depending upon how much of the drop was really excess, and upon the thoroughness of the flocking out of AgCl). Its disappearance is due to the reaction between the sulfocyanate and the very finest of the suspended particles of silver chloride. If another drop is added, the color will persist several minutes, for the sulfocyanate must then react with silver chloride particles of larger size and smaller aggregate surface. If still another drop is added the color will persist from 15 minutes to an hour or more (even for 19 hours),

TABLE II.

Centrifuge time.	Persistence of color ($Fe(CNS)_3$).			
	End-point.	Excess drops of KCNS solution.		
		First.	Second.	Third.
min.				
None.	40 sec.	4 min.	15 min.	More than 1 hr.
1	90 "	24 "	More than 1 hr.	
6	75 "	23 "	" " 1 "	
40	30 min.	More than 1 hr.		

for the fine suspension of silver chloride has all been used up, and the aggregate surface left for reaction is very small.

The influence of the silver chloride particles of different sizes can be demonstrated by fractional centrifugalization, as in the experiments given in Table II.

In addition to the action of nitric acid in flocking out the chloride, the abundance of ferric alum used also retards the reaction between silver chloride and sulfocyanate by reducing the ionization of the latter. This also deepens the end-point color by preventing the ionization of the red salt, $Fe(CNS)_3$, into yellow Fe^{+++} ions and colorless CNS^- ions.

II. There remains the second question: "Does the amount of silver precipitation, as found by titration of the excess, indicate accurately the chlorides of the plasma?"

Mixtures of tungstic acid and chloride bring down more silver than can be accounted for by the chloride alone. This additional precipitation of silver has been found by centrifugalization and titration of the supernatant fluid. Errors as large as 4 per cent were obtained in this way. Similar errors sometimes occurred when centrifugalization was introduced into the chloride determination of tungstic acid filtrates. The amount of error which may so occur is not always proportional to the amount of tungstic acid present, and so appears to be more probably dependent upon physical than upon chemical reactions.

This possibility of error is, however, entirely avoided in the method described, by carrying on the titration in the presence of the precipitate, when all the silver which has not been truly precipitated by chloride is available for titration. Determinations of the same 0.1 M NaCl solution gave practically identical results whether determined directly or after the addition of equal volumes of 10 per cent sodium tungstate solution and $\frac{2}{3}$ N sulfuric acid; for example, with tungstate, 3.53, 3.54, and 3.54 mg. of Cl per cc., and without tungstate, 3.547 as the average of eleven determinations.

The evidence given above has been presented in order to demonstrate that the method is free from error at the two points which seemed, *a priori*, the most probable sources; namely, the presence of tungstic acid at the time silver chloride is precipitated, and the presence of silver chloride at the time of titration.

Checks.

The final test of the accuracy of the method consists of course in its comparison with methods of known accuracy. The check determinations are given in Table III.

These check determinations indicate the essential accuracy of the method described. In order to determine the limit of error, seventeen duplicate determinations, involving nine separate precipitations with tungstic acid, were made on the same plasma, with the following results: 3.56; 3.51; 3.52; 3.54; 3.50; 3.55; 3.57; 3.54; 3.48; 3.49; 3.52; 3.50; 3.52; 3.53; 3.56; 3.54; 3.55 mg. of Cl per cc. The digestion method of von Korányi showed 3.53 mg. of Cl per cc. The average by the method described is 3.528. The

TABLE III.

Specimen.	Method described.		NaCl checked by method of
	Cl per cc.	NaCl	
	mg.	*per cent*	*per cent*
			Foster.*
Whole blood. M.	2.88	0.473	0.481
	2.88	0.473	0.479
Plasma. M.	3.69	0.608	0.619
	3.71	0.612	0.617
Whole blood. Wh.	3.06	0.503	0.504
Plasma. Wh.	3.84	0.633	0.635
			McLean-Van Slyke.†
Plasma I.	3.76	0.619	0.633‡
" II.	3.78	0.623	0.618‡
" III.	3.75	0.618	0.609‡
" IV.	3.74	0.617	0.606‡
			von Korányi.§
" V.	3.68	0.606	0.603
" VI.	3.80	0.626	0.619
" VII.	3.52	0.580	0.584
" VIII.	3.80	0.626	0.631
" IX.	3.52	0.582	0.582
Whole blood I.	3.24	0.535	0.542
Ascitic fluid I.	4.10	0.676	0.677
" " II.	3.78	0.623	0.625
			Bell and Doisy.‖
Plasma X.	3.75	0.619	0.614‡
" XI.	3.38	0.558	0.567‡
" XII.	3.64	0.600	0.596‡
" XIII.	3.70	0.610	0.605‡
" XIV (diluted).	2.84	0.467	0.465‡
Plasma XV.	3.86	0.637	0.641‡
Average		0.5885	0.5896

* Foster, G. L., A modification of the McLean-Van Slyke method for the determination of chlorides in blood, *J. Biol. Chem.*, 1917, xxxi, 483. (Allowance for end-point has been made, as introduced by Van Slyke and Donleavy (Van Slyke, D. D., and Donleavy, J. J., *J. Biol. Chem.*, 1919, xxxvii, 551).

† McLean, F. C., and Van Slyke, D. D., A method for the determination of chlorides in small amounts of body fluids, *J. Biol. Chem.*, 1915, xxi, 361.

‡ I am indebted to Mr. L. M. Smith for these determinations.

§ von Korányi, A., Physiologische und klinische Untersuchungen über den osmotischen Druck thierischer Flüssigkeiten, *Z. klin. Med.*, 1897, xxxiii, 1.

‖ Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, 1920–21, xlv, 427.

greatest deviations are −1.3 and +1.2 per cent. The limit of error with careful technique must therefore be less than 1.5 per cent.

SUMMARY.

1. A simple and rapid method is described for the determination of blood and plasma chlorides.
2. The same reagents as are used for the determination of urine chlorides are employed.
3. The method is especially adapted to the system of blood analysis developed by Folin and Wu.
4. The limit of error is less than 1.5 per cent.

The writer is indebted to Professor Otto Folin for encouragement in this work, and to Mr. L. M. Smith for assistance in checking determinations.

THE DETERMINATION OF CHLORIDES IN BLOOD PLASMA.

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(Received for publication, December 6, 1920.)

Van Slyke and Donleavy (1919) have published a method for the determination of chlorides in blood plasma, in which both proteins and Cl are precipitated together by a solution containing picric acid, nitric acid, and standard silver nitrate. The excess silver was titrated in the filtrate by the iodometric method of McLean and Van Slyke (1915). The results in a series of normal human plasmas were identical with those obtained by the method of McLean and Van Slyke, which involved two successive precipitations and filtrations; one of the proteins, the other of the silver chloride. It seemed, therefore, that the Van Slyke-Donleavy method, which involved only one precipitation and filtration, constituted a desirable simplification in the technique for determining plasma chlorides, although it was found still necessary to use the double precipitation for chlorides in whole blood (Austin and Van Slyke, 1920).

We have in the meantime tested the single precipitation method on plasmas from pathological cases by comparing the results with those of the double precipitation method used by Austin and Van Slyke on whole blood, and with those of the total chlorine determination by the Carius method (Table I). In the double precipitation method the preliminary precipitation of the proteins was performed as described by Austin and Van Slyke, except that for 3 cc. of plasma only 10 cc. of picric acid solution are used, instead of the 30 cc. used for 3 cc. of whole blood. The larger amount of picric acid is not needed for plasma, and if employed may result later, when nitric is added, in an undesirable separation of picric acid crystals. The Carius determination was carried out as described by Austin and Van Slyke.

TABLE I.

No.	Appearance of oxalate plasma.	Condition of donor.	Chloride, calculated as NaCl, per liter of plasma.					
			Carius method.		Double precipitation method.		Single precipitation method.	
			Separate determinations.	Average.	Separate determinations.	Average.	Separate determinations.	Average.
			gm.	gm.	gm.	gm.	gm.	gm.
1	Clear.	Normal.	6.02 5.97 6.16 6.15	6.08	6.08 6.08	6.08	6.07 6.08	6.08
2	"	"	6.23 6.31	6.27	6.24 6.24	6.24	6.32 6.30	6.31
3	"	"	6.09 6.13	6.11			6.07 6.02	6.05
4	"	"	6.06 6.06 6.08	6.07	5.98 6.07	6.03	6.18 6.08	6.13
5	Deep amber.	Nephritic.	5.29 5.31	5.30	5.29 5.31	5.30	5.48 5.49	5.49
6	" "	Cardiac.	5.87 5.97 5.87 5.97	5.92	5.86 5.86	5.86	6.05 6.03	6.04
7	Amber.	Nephritic.	5.87 5.91 5.92	5.90	5.95 5.95	5.95	6.09 6.19	6.14
8	Creamy opaque.	"			6.02 5.94	5.98	6.07 6.08	6.08
9	Clear.	Osteomyelitis.	5.76 5.76 5.76 5.64	5.73	5.72 5.68	5.70	5.88 5.85	5.87
10	"	Arteriosclerosis.	6.06 6.06 6.08	6.07	5.98 6.03	6.01	6.08 6.18	6.13

CONCLUSIONS.

We have encountered plasma specimens from hospital patients in which the single precipitation gave results indicating a higher chloride content than that obtained when a preliminary removal of the proteins was performed. When such disagreement occurred, the results by the Carius method confirmed those by the double precipitation method of Austin and Van Slyke.

Since the factors which interfere with the single precipitation method in pathological plasmas are not known, it is desirable in all plasmas, both normal and pathological, to remove the proteins by a preliminary precipitation, as in the Austin-Van Slyke method for whole blood, before the chlorides are precipitated with standard silver nitrate.

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McLean, F. C., and Van Slyke, D. D., *J. Am. Chem. Soc.*, 1915, xxxvii, 1128; *J. Biol. Chem.*, 1915, xxi, 361.

Van Slyke, D. D., and Donleavy, J. J., *J. Biol. Chem.*, 1919, xxxvii, 551.

AN IMPROVED APPARATUS FOR USE IN FOLIN AND WU'S METHOD FOR THE ESTIMATION OF UREA IN BLOOD.

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(Received for publication, December 21, 1920.)

In carrying out many determinations of urea in blood by the method of Folin and Wu[1] we encountered much difficulty in preventing frothing while distilling over the ammonia. Many of the usual antifoaming mixtures were tried with little success.

The apparatus described below was devised to overcome this difficulty and has been found to prevent entirely any liquid being carried over as froth into the receiving tube.

A glass tube with a bulb in the center (a 25 cc. pipette) is bent as shown in Fig. 1. A number of small holes are blown in the side, and a constriction is made at the end, of the tube from which distillation proceeds.

The large bubbles of foam, in passing through the numerous small holes, are broken up and the bulb in the tube further safeguards any possibility of liquid being carried over into the receiver.

This apparatus, somewhat enlarged, makes a perfect substitute for the distilling bulb used in the ordinary Kjeldahl determination of nitrogen.

[1] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81.

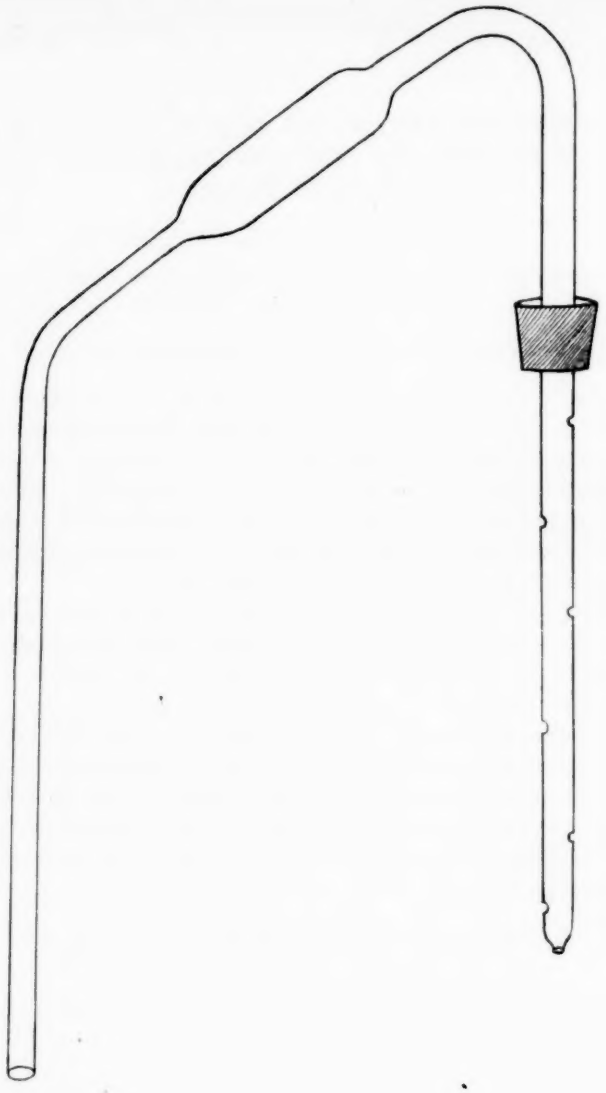

FIG. 1. Apparatus for use in 'urea 'determinations by the method of Folin and Wu.

THE CHEMICAL STRUCTURE OF CHONDRIDIN.*

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(Received for publication, December 31, 1920.)

Hebting,[1] working in Hofmeister's laboratory, has obtained on hydrolysis of chondroitin sulfuric acid a crystalline product which he named chondridin. Hebting recognized that the substance was related to chondrosin, but found that it differed from the latter in its elementary composition and its capacity to crystallize.

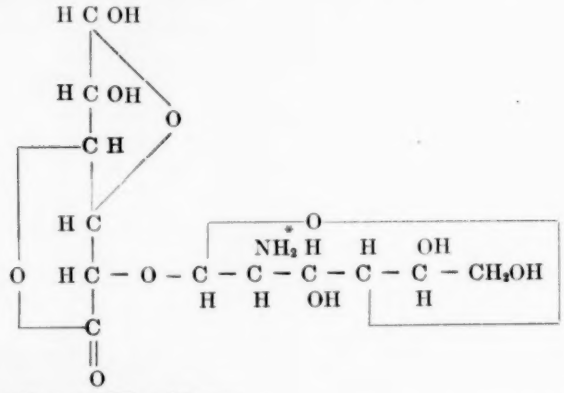
The structural representation of chondroitin sulfuric acid as formulated by Schmiedeberg, and later by Levene and La Forge, admits in the organic radical of the molecule the presence of only one derivative of chondrosin; namely, of its acetyl derivative. It therefore seemed probable that chondridin was not a primary decomposition product, but one formed from chondrosin through manipulation. This hypothesis was borne out by the present investigation. Hebting has found that the composition of chondridin differed from chondrosin by the presence in the molecule of a surplus of hydrogen and oxygen equivalent to 1 molecule of water.

A simple explanation of this difference may be given by assuming that chondridin consists of unchanged chondrosin containing a molecule of crystal water. On the other hand this assumption was scarcely adequate to explain the great difference in the capacity for crystallization of the two substances. More adequate seemed the assumption that chondridin was a lactone of chondrosin, crystallizing with crystal water. The substance

* I regret that the article of Schmiedeberg (Schmiedeberg, O., *Arch. exp. Path. u. Pharmakol.*, 1920, lxxxvii, 47) had escaped my attention until after this work was prepared for publication. The present communication incidentally contains an answer to his criticism on my theory of the place of linkage of chondrosamine and of glucuronic acid. Other criticisms of Professor Schmiedeberg have been met in previous publications. —P. A. L.

[1] Hebting, J., *Biochem. Z.*, 1914, lxiii, 353.

analyzed by Hebting apparently contained 2 molecules of crystal water. The samples analyzed by us seemed to contain $2\frac{1}{2}$ molecules. The graphic representation of the anhydrous substance would then be as follows:



* Allocation of NH_2 arbitrary.

molecules of crystal water is $C_{12}H_{19}NO_{10} + 2\frac{1}{2}\ H_2O$.

The experimental evidence to be reported in this communication seems to support the second hypothesis.

The lactone structure of the substance was suggested by the method of preparation and was confirmed by the result of the titration of the aqueous solution with alkali. When to a cold solution of the substance 0.1 N alkali is added rapidly, the solution reacts neutral after the addition of 1 drop of the alkali (alizarin being used as indicator). When to the aqueous solution of the substance the alkali was added in excess, and when the alkaline solution was titrated back after standing 18 hours, it was found that 0.1000 gm. of the substance neutralized 2.55 cc. of 0.1 N alkali. On this basis the molecular weight is calculated to 382, while the theory for $C_{12}H_{19}NO_{10} + 2\frac{1}{2}\ H_2O$ is 392. Thus, of the two assumptions that of the lactone structure of the substance seems the more acceptable.

The substance reduces Fehling's solution, has its primary amino group unsubstituted, and yields a quantity of furfurol which approximates the one required by theory for chondrosin.

The air-dry substance on drying under diminished pressure at the temperature of water vapor loses $1\frac{1}{2}$ molecules of crystal

water. If the lactone structure of the substance is correct one has to accept that under the given conditions of drying the resulting substance still retains 1 molecule of crystal water.

EXPERIMENTAL PART.

The mode of preparation of the substance differed in its details from that of Hebting. The analytical data published by this author for chondridin seemed to agree with those required by theory for chondrosin oxalate. At the outset of the work it was planned to test this possibility. Chondrosin chlorohydrate was prepared following the conditions employed in the earlier work by Levene and La Forge. The chondrosin chlorohydrate obtained in this manner was freed from hydrochloric acid. To the aqueous solution of chondrosin a slight excess over one equivalent of oxalic acid was added and to the solution alcohol was added to opalescence. On standing there was no evidence of crystallization. However, when the solution was allowed to stand on a boiling water bath for an hour prior to the addition of alcohol, crystallization did take place. Alcohol was added to marked opalescence and the solution was allowed to remain on the water bath until it clarified. On scratching along the walls of the beaker a crystalline deposit soon began to form. The substance was recrystallized by dissolving in hot water, adding to the aqueous solution 99.5 per cent alcohol to opalescence, and boiling the solution until it clarified. After three or four recrystallizations the substance contained only traces of mineral impurities.

In later experiments the preparation of the substance was somewhat simplified; namely, no attempt was made to isolate the chondrosin hydrochloride before the digestion with oxalic acid. The procedure was as follows. Portions of 50.0 gm. of the barium salt of chondroitin sulfuric acid were hydrolyzed by heating for 1 hour on the water bath in a solution of 150 cc. of 20 per cent hydrochloric acid. From the product of reaction barium and hydrochloric acid were removed and the solution was concentrated to a small volume under diminished pressure. The subsequent treatment was as above described.

Properties of the Substance.—The lactone is a white crystalline powder. It does not melt, but contracts and turns dark at 200°C. The analysis of the substance was as follows:

1. 0.1176 gm. of the substance (No. 43) dried to constant weight at the temperature of water vapor and under diminished pressure lost 0.0078 gm.

2. 0.1130 gm. of another sample (No. 81) under the same conditions lost 0.0075 gm.

	Calculated for $C_{12}H_{21}NO_{11} + 1\frac{1}{2}\ H_2O$. *per cent*	Found. *per cent*
H_2O	7.20	6.63

1. 0.1098 gm. of the above substance gave on combustion 0.1626 gm. of CO_2 and 0.0580 gm. of H_2O.

0.1867 gm. of the substance used for Kjeldahl nitrogen estimation required for neutralization 5.3 cc. of 0.1 N acid.

For the amino estimation 0.050 gm. of the substance was dissolved in 5 cc. of water.

2 cc. of the solution in the Van Slyke micro apparatus gave 1.36 cc. of nitrogen gas at $T = 20°C.$ and $P = 764$ mm.

2. 0.1055 gm. of the substance gave 0.1564 gm. of CO_2 and 0.0568 gm. of H_2O.

	Calculated for $C_{12}H_{19}NO_{10} + H_2O$. *per cent*	Found. No. 1. *per cent*	Found. No. 2. *per cent*
C	40.54	40.43	40.11
H	5.96	5.98	5.92
N	3.94		3.97
NH_2 N	3.94		3.89

The rotation of the air-dry substance was as follows:

$$[\alpha]_D^{20} = \frac{+0.97 \times 100}{1.6 \times 1} = +60.6°$$

Titration of the Substance with Alkali.—0.1000 gm. of the substance was dissolved in 25 cc. of water and titrated with 0.1 N sodium hydroxide. Alizarin was used as indicator. After the addition of the first drop the solution reacted neutral. 0.1000 gm. of the substance was dissolved in 25 cc. of water. 15 cc. of 0.1 N alkali were added and the solution was allowed to stand over night. It required 12.45 cc. of 0.1 N acid to titrate the solution to neutral.

	Calculated for $C_{12}H_{19}NO_{10} + 2\frac{1}{2}\ H_2O$.	Found.
Molecular weight	382.17	392

Furfurol Estimation.—0.2000 gm. of the substance was distilled in the usual way with hydrochloric acid having a specific gravity of 1.06. The yield of the phloroglucide was 0.0248, which corresponds to 0.00744 gm. of glucuronic acid.

	Calculated for $C_{12}H_{19}NO_{10} + 2\frac{1}{2}\ H_2O$.	Found.
	per cent	*per cent*
Glucuronic acid	50.77	37.2

Taking into consideration the limit of error of the method the result is not unsatisfactory.

SULFOCYANATE CONTENT OF THE SALIVA AND URINE IN PELLAGRA.

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(Received for publication, December 27, 1920.)

I. The Sulfocyanate Content of the Saliva.

In previous work on the saliva of pellagra patients[1] at the United States Pellagra Hospital, Spartanburg, South Carolina, it was found that the sulfocyanate reaction with ferric chloride and dilute hydrochloric acid was much less marked for the salivas of the pellagra patients than for normal people.

Subsequent to the general survey of the saliva and the testing of the saliva with ferric chloride, Sullivan and Jones, using the very accurate Rupp-Schied[2] method, improved by Thiel[3] made quantitative estimations of the sulfocyanate content of the saliva of seven patients who were somewhat improved as regards general physical condition and extent of erythema and dermatitis. The sulfocyanate content of the saliva of these seven cases estimated as KSCN varied from 8 to 25.8 parts per million with an average of 18.6 parts per million. These quantitative findings were utilized by Sullivan and Jones as a basis for the conclusion[4] that the sulfocyanate content of patients, even in a convalescing stage, was less than normal.

In 1919 the study of the sulfocyanate content of the saliva of pellagra patients was again taken up. The type of case admitted to the hospital in 1919, however, was somewhat different from those of 1917. In 1917 the patients admitted were rather marked

[1] Sullivan, M. X., and Jones, K. K., *Public Health Rep., U. S. P. H.*, 1919, xxxiv, 1068.

[2] Rupp, E., and Schied, A., *Ber. chem. Ges.*, 1902, xxxv, 2191.

[3] Thiel, A., *Ber. chem. Ges.*, 1902, xxxv, 2766.

[4] Sullivan and Jones,[1] p. 1076, foot-note 5.

cases of pellagra on the whole, while in 1919 the cases were of a milder type and in better physical condition. The difference in the two sets of patients held for the saliva. Thus in 1917, out of 40 cases tested in the hospital, the saliva of 32 gave no perceptible pink or red color with ferric chloride with or without the addition of hydrochloric acid, while in 1919, of 66 cases so tested, 47 were faintly or strongly positive. Thus, crude as the ferric chloride test admittedly is, it showed difference between the salivas of the 1917 patients and of the 1919 patients.

Two quantitative methods had been tested in the laboratory. Munk's[5] gravimetric method and the Rupp and Schied[2] method improved by Thiel.[3] In the Munk method, the sulfocyanate is precipitated by silver nitrate in the presence of dilute nitric acid and the silver precipitate melted with sodium carbonate and potassium nitrate to convert the sulfur of the sulfocyanate to sulfate. From the sulfur in the sulfate precipitated by barium chloride the sulfocyanate is calculated. The Rupp and Schied method improved by Thiel is an iodometric method.

The method is based on the fact that sulfocyanate in the presence of excess of bicarbonate decolorizes large amounts of iodine solution as illustrated by the following equation.

$$KSCN + 4\,I_2 + 4\,H_2O = H_2SO_4 + 6\,HI + KI + CNI$$

The process is completed in the course of 4 hours at ordinary temperature. Then with careful acidification with hydrochloric acid[3] hydriodic acid is liberated and the cyanogen iodide decomposed.

$$HI + CNI + HCN = I_2$$

The entire process can be expressed as follows:

$$KSCN + 3\,I_2 + 4\,H_2O = H_2SO_4 + 5\,HI + KI + HCN$$

1 molecule of sulfocyanate is thus equivalent to 6 atoms of iodine as iodide.

The Rupp-Schied-Thiel method, which was found very satisfactory by Gies and his collaborators[6] in their extensive study of sulfocyanate in the mammalian body, was preferred by us to the Munk method. For the saliva work done at the hospital the procedure is given below.

After thoroughly rinsing the mouth with water the saliva was collected under the stimulus of chewing white paraffine wax. Obviously, it was not possible to get 24 hour samples of saliva so a sample collected for a definite length of time, in most cases 30 minutes, was used for comparison. This sample was collected between 10 and 11 a.m. or approximately 1½ hours before the noon meal. The samples collected shortly after the pa-

[5] Munk, I., *Virchows Arch. ges. Physiol.*, 1877, lxix, 350.

[6] Gies, W. J., and Kahn, M., *Dental Cosmos*, 1913, lv, 40; Gies, W. J., Lieb, C. C., and Kahn, M., *Dental Cosmos*, 1914, lvi, 175.

tient's entrance to the hospital and shortly before discharge were compared. The saliva was expectorated into a beaker, while the subject directed his entire attention to the collection of saliva.

The specimens thus obtained were carefully measured, ranging in volume from 10 to 100 cc., and transferred to suitably sized Erlenmeyer flasks. The mucin was precipitated by treatment with 1 per cent acetic acid and moderate heating on a hot plate; and the coagulated matter, as well as any other solid material present, was removed by filtering through folded filters. It was discovered that salivas vary widely in the ease with which this precipitation is effected and in some cases filtration was rendered difficult through plugging of the filter with colloidal matter. The residue and filters were thoroughly washed with warm water to remove any traces of $-CNS$ that might be retained thereon; and the combined filtrates and washings were transferred to 200 cc. volumetric flasks and made up to volume. In later work it was found advisable to add a little 95 per cent alcohol to these preparations before making up to volume. The addition of a small amount of alcohol in no way interfered with the subsequent determinations and prevented the development of microorganisms, in case the material was to stand any length of time before analysis, and aided in preventing the development of colloidal precipitates when treated with silver nitrate in the next stage of the process.

Of the 200 cc. preparations, definite quantities, in most cases 75 cc., were measured with a pipette into moderate sized beakers for duplicate determinations. The material was acidified with dilute HNO_3 and the $-CNS$ precipitated as the silver salt by addition of an excess of $AgNO_3$. Owing to the already great dilution, it was found more satisfactory to add the $AgNO_3$ in the form of a concentrated solution, approximately 25 per cent, drop by drop, accompanied by thorough agitation with a glass rod, until a slight excess was reached, instead of using the dilute 3 per cent solution generally employed with other types of material. To facilitate sedimentation a small amount of infusorial earth (previously calcined, washed in HNO_3, and dried) was added, thoroughly stirred in, and the solution placed upon a boiling water bath for about 10 minutes. Filtration was effected by means of suction through an ordinary filter paper on a platinum cone. The filtrate should be perfectly clear. If not, and this frequently occurred, it was passed through the filter again after the addition of another small amount of infusorial earth, the process being repeated until an absolutely clear filtrate was obtained. At this stage in the process some salivas gave a good deal of trouble on account of the formation of colloidal precipitates after the addition of $AgNO_3$, making them difficult or practically impossible to filter. However, with few exceptions, it was found that repeated passing through the filter and liberal use of infusorial earth would result in complete separation of the material. The contents of the beaker were thoroughly washed out into the filter, using a rubber "policeman," and the precipitate on the filter was likewise thoroughly washed with 1 per cent HNO_3.

The filter and precipitate were then transferred to a wide necked Erlenmeyer flask of 1 liter capacity, 3 gm. of $NaHCO_3$, 3 gm. of KI, and a little water added, and the whole was stirred until the filter paper was completely disintegrated. In practice it was found more convenient to employ solutions of KI and $NaHCO_3$ and add volumes of each sufficient to provide the equivalent of 3 gm. in each case. The KI is added to convert any chlorides present to iodides and thus prevent reaction with the standard iodine solution added later; 3 gm. were found more than sufficient for the quantities encountered in this work.

To the mixture was now added, quantitatively, standard iodine solution until the brown color persisted after several minutes gentle agitation. A standardized iodine solution of 0.05 N strength was used and 10 cc. of this, measured out with a calibrated pipette, were found to be sufficient to provide excess in any of the material worked with. The advantage of adding a uniform quantity, eliminating difficult burette readings, is obvious. The flask was now stoppered tightly with a paraffined cork and set away in a dark place at room temperature for a period of 4 or 5 hours, which is sufficient time for completion of the reaction at ordinary temperature.

At the expiration of this time the mixture was acidified with 10 per cent HCl, using caution to avoid too rapid evolution of CO_2 and hence risk loss of volatile iodine; a few cc. of dilute starch paste (about 3 per cent) were added; and the excess of iodine was titrated with a standard 0.05 N sodium thiosulfate solution. The difference between the amount of 0.05 N iodine originally added and the excess thus determined represents the iodine which has reacted with the −CNS. Since, as shown previously, 1 molecule of −CNS corresponds to 6 atoms of iodine under such conditions, 1 cc. of 0.05 N iodine corresponds to 0.81015 mg. of −CNS, expressed as KCNS, or 1 cc. of 0.1 N iodine corresponds to 1.6203 mg. of KCNS. Likewise the number of cc. of iodine reacted multiplied by the factor 0.81015 for 0.05 N or 1.6203 for 0.1 N will give the quantity of −CNS expressed in mg. of KCNS.

It was found that this method, carried out as just described, gave uniformly accurate results even with the small quantities of material sometimes available for analysis and with the small concentration of −CNS in many samples, provided proper precautions were taken to insure absolutely correct standardization of solutions and to correct for marked changes in temperature or other conditions. Determinations made upon a pure solution of KCNS of known strength comparable to the quantity of sulfocyanate calculated as KSCN in the saliva, gave results with an error of less than 1 per cent.

Though it has not been proved that the sulfocyanate exists in the body as a potassium salt, in our work it is always computed

as KSCN. The results with the saliva of the Pellagra Hospital patients are given in Tables I and II. In Table I the results

TABLE I.

Sulfocyanate Content of Saliva Collected in 30 Min.

Status of pellagra at time of testing.					
Active.		Convalescent.		Normal.	
Case No.	K S C N	Case No.	K S C N	Case No.	K S C N
1919	*mg.*		*mg.*		*mg.*
579	0.88	555	1.41	1	1.82
586	3.53	556	1.55	2	1.95
10*	0.50	557	0.69	3	2.43
11	1.05	563	2.20	4	1.35
12	1.67	567	4.36	5	1.37
15	1.01	572	1.24		
16	2.67	577	1.36		
19	0.49	582	3.70		
20	0.63	586	8.18		
21	1.90	11	4.79		
22	0.61	12	4.36		
23	1.07	15	1.35		
24	0.89	16	3.66		
26	1.61	19	1.25		
28	1.80	20	0.90		
29	1.28	21	2.13		
30	1.36	22	1.61		
31	1.89	23	1.47		
32	2.21	24	2.24		
33	0.39	26	2.56		
34	1.04	28	1.08		
		29	1.28		
		30	5.14		
		31	3.32		
		32	3.57		
		33	0.93		
Average..	1.36		2.55		1.78

*A new series of case numbers was started July 1.

from all patients tested are given. In Table II only those cases where the saliva was tested within a few days after admission and also within a few days of their discharge are presented.

With the exception of Case 28, which gives a lower value for the sulfocyanate content of the saliva at discharge than at entrance, and Case 29 with the same sulfocyanate content at entrance and at discharge, the sulfocyanate content of the saliva is lower at entrance than at discharge from the hospital.

Table II shows that the increase of the sulfocyanate is not proportional to the increase in volume of saliva. Thus in three

TABLE II.

Comparison of K S C N in Saliva at Admission and at Discharge.

Case No.	First test.			Last test.		
	Volume of saliva.	K S C N in sample.	K S C N per 100 cc. saliva.	Volume of saliva.	K S C N in sample.	K S C N per 100 cc. saliva.
	cc.	*mg.*	*mg.*	*cc.*	*mg.*	*mg.*
586	35	3.53	10.09	66	8.18	12.40
11	54	1.05	1.94	95	4.79	5.04
12	36	1.67	4.64	54	4.36	8.07
15	50	1.01	2.02	50	1.35	2.70
16	84	2.67	3.18	87	3.66	4.21
19	49	0.49	1.00	55	1.25	2.27
20	40	0.63	1.58	59	0.90	1.53
21	60	1.90	3.17	38	2.13	5.61
22	48	0.61	1.27	62	1.61	2.60
23	71	1.07	1.51	70	1.47	2.10
24	55	0.89	1.62	49	2.24	4.57
26	48	1.61	3.35	55	2.56	4.65
28	60	1.80	3.00	47	1.08	2.30
29	25	1.28	5.12	51	1.28	2.51
30	24	1.36	5.67	65	5.14	7.91
31	21	1.89	9.00	39	3.32	8.53
32	53	2.21	4.17	78	3.57	4.58
33	45	0.39	0.87	39	0.93	2.38
Average.....	47.7	1.45	3.51	58.8	2.77	4.66

cases (Nos. 15, 16, and 23) the volume is the same while the KSCN content is increased. In three other cases (Nos. 21, 24, and 33) the volume is decreased while the KSCN is increased. For Case 28 there is at discharge a decrease both in volume of the saliva collected in 30 minutes and in the sulfocyanate content thereof. Case 29 has an increase in volume at discharge with no variation in the sulfocyanate content. In ten cases there is an

increase in both the volume and the KSCN content of the saliva of the patients about to be discharged as compared with the saliva at entrance, but the increase in the KSCN content is proportionately very much greater. Thus the average volume of these ten cases (Nos. 586, 11, 12, 19, 20, 22, 26, 30, 31, and 32) is 40.8 cc. at entrance and 62.8 cc. at discharge, while the corresponding content of KSCN is 1.51 and 3.57 mg. The same conclusion holds for the saliva as a whole; namely, that the sulfocyanate is increased at discharge to a much greater degree than the volume.

Calculated on the basis of 100 cc. of saliva, the sulfocyanate is higher at discharge than at entrance in fifteen of the eighteen cases given in Table II. For Case 20 the sulfocyanate calculated to 100 cc. is slightly lower at discharge than at entrance, while for Cases 28 and 29 it is distinctly lower. Still, as a rule, the percentage of sulfocyanate in the saliva is higher at discharge than at entrance to the hospital.

The sulfocyanate content of the saliva as given in the literature is rather variable, due, perhaps, to different methods. Munk[5] using his own method found the saliva of man to contain 0.014 per cent of NaSCN (0.0167 per cent KSCN); Bruylants[7] found a trace to 0.0698 gm. of HSCN per liter, with an average for forty-five cases of 0.0374 gm. of HSCN (or the equivalent of 0.0062 per cent of KSCN). Krüger[8] using Munk's method found 0.0072 per cent of KSCN in the saliva; Mayer[9] using the Rupp-Schied-Thiel method found 0.0003 per cent of KSCN in the saliva; while Gies and Kahn,[6] using the same method, found 12.8 mg. of KSCN in 500 cc. of saliva.

The only reference to the saliva in pellagra that we have found is that by Mense,[10] who tested the saliva of pellagrins by means of a pad of test paper presumably impregnated with starch and iodic acid, which permits judging of the content in KSCN by the formation of a blue color following the release of iodine. Of twenty-nine pellagrins or suspects selected, seven showed absence

[7] Bruylants, J., *Bull. Acad. méd., Belgique*, 1888, ii, series iv, 21.

[8] Krüger, F., *Z. Biol.*, 1899, xxxvii, 6.

[9] Mayer, in Oppenheimer, C., Handbuch der Biochemie des Menschen und der Tiere, Berlin, 1908, iii, pt. i, 33.

[10] Mense, C., *Arch. Schiffs. u. Tropenhyg.*, 1913, xvii, 788.

of KSCN; eight a very feeble reaction (about 0.001 per cent); seven a feeble reaction (0.003 per cent); and only six a normal reaction (0.01 per cent).

As the excretion of sulfocyanate seems to be somewhat of an individual question, we believe the best control on the pellagra patient is the same patient recovered to such a degree that all the signs and symptoms characteristic of pellagra have disappeared. Accordingly stress is laid on the difference in the sulfocyanate of the saliva as found at entrance and at discharge. Of the eighteen cases of Table II, the saliva of sixteen increased in sulfocyanate during their stay in the hospital.

TABLE III.

Standard Diet for 1919.

Diet 1.	Diet 2.
Soda biscuits.	Soda biscuits.
Corn bread.	Corn bread.
Rice.	Rice.
Tomatoes.	Irish potatoes.
Orange or evaporated apples.	Grits.
Grits.	Gravy.
Mush.	Syrup.
Gravy.	Corn-meal mush.
Cheese.	Milk.
Veal, beef, chicken, or fish.	
Milk.	

In actual average figures, the KSCN content of the saliva shortly after admission of the patient was 1.45 mg. in the sample collected, with a percentage of 0.00351, while at discharge it was 2.77 mg. and 0.00466 per cent. The sulfocyanate content of the saliva was, as a rule, increased at discharge despite the fact that, barring the possible difference of utilization, the diet at entrance and shortly thereafter was more varied and potentially could yield more HSCN than the latter diet.

At entrance the diet given the patients contained cereals, vegetables, meat or fish, and milk. In a short time the meat and fish were eliminated, and as much milk as the patient would take, usually 1,000 to 1,800 cc. a day, was given. Examples of the diets used are given in Table III. No measurement of quantity

was made aside from the measurement of the milk. Diet 1 is the diet used for a short time after entrance; Diet 2 the diet used for most of the time the patient was in the hospital until discharge.

II. The Sulfocyanate Content of the Urine.

As the sulfocyanate of the saliva was found in general to increase as the patients passed from the pellagrous stage to the convalescent stage, attention was paid to the sulfocyanate content of the urine. The patients available for the urine study were rather mild cases of pellagra. The diet employed during the last tests just before discharge was Diet 2 of 1919, which was less varied than Diet 1 and relied upon milk as the main source of nitrogen. Still of the fourteen cases given in Table IV, eleven gave a higher content of KSCN in the 24 hour urine at discharge than at entrance. In two cases (Nos. 26 and 32), the increase was slight. In three cases (Nos. 28, 30, and 31), there was a reduction in sulfocyanate at discharge as compared with entrance. The average of the fourteen urines studied comparatively, however, is higher for the absolute amount of KSCN at discharge than at entrance.

Of the fourteen cases given in Table IV, the volume of the urine is increased at discharge in twelve cases; in two (Nos. 15 and 28) it is decreased. As the volume of the urine of the patients at discharge, when they showed no signs or symptoms, which in the judgment of the medical staff indicate pellagra, is on the whole decidedly increased, the percentage of sulfocyanate in the urine, that is the quantity in 100 cc. of urine, is as a rule less at discharge than at entrance. Thus in ten cases (Nos. 16, 19, 20, 21, 26, 29, 30, 31, 32, and 33), the percentage of sulfocyanate in the urine is reduced while in only four cases (Nos. 15, 23, 24, and 28) is it increased. It may be emphasized, however, that the absolute amount of sulfocyanate excreted in the 24 hour urine, is, as a rule, increased at discharge.

In Table V are given data to show that, as a rule, the same order obtains with both saliva and urine with both collected within the same 24 hours; that is, that the milligrams of KSCN in the saliva and in the 24 hour urine are, as a rule, greater at discharge than at entrance.

It may be noted, however, that of the eleven cases given in Table V, the sulfocyanate content of the saliva is reduced at discharge in two cases (Nos. 28 and 29), and that the sulfocyanate content of the urine is reduced at discharge in two cases (Nos. 28 and 30). However, the fact obtains that in general for both saliva and urine, when taken within the same 24 hours, the sulfocyanate content is increased, as a rule, at discharge—a corroboration of the data given for the saliva and the urine in Tables II and IV, respectively.

TABLE IV.

K S C N of the 24 Hr. Urine.

Case No.	Active pellagra.			Convalescent.		
	Volume.	K S C N in urine.	K S C N in 100 cc.	Volume.	K S C N in urine.	K S C N in 100 cc.
	cc.	*mg.*	*mg.*	*cc.*	*mg.*	*mg.*
15	1,220	34.1	2.80	1,120	43.3	3.87
16	680	50.0	7.35	1,650	94.2	5.71
19	1,230	60.9	4.95	1,955	67.3	3.44
20	1,325	79.6	6.00	2,240	85.3	3.81
21	640	51.8	8.09	1,400	68.6	4.90
23	530	26.1	4.92	1,115	76.4	6.85
24	1,470	15.7	1.07	1,690	58.2	3.44
26	1,530	52.9	3.46	1,960	55.1	2.81
28	2,965	78.2	2.64	1,770	47.9	2.71
29	455	45.8	10.07	1,390	62.0	4.46
30	1,000	61.8	6.18	1,600	43.3	2.71
31	1,420	52.2	3.68	1,830	44.2	2.42
32	930	93.5	10.05	1,260	96.6	7.67
33	550	49.7	9.04	1,230	54.1	4.40
Average.....	1,139	53.7	5.74	1,586	64.0	4.23

Fenwick[11] believes that sulfocyanate as a product of protein metabolism affords indirect evidence of the extent of the nitrogenous metabolism of the organism and that its amount diminishes under conditions in which the activity of the nutritive function is diminished.

According to Grober,[12] sulfocyanate excretion is very slight in the case of cachetic and sick people.

[11] Fenwick, S., The saliva as a test for functional disorders of the liver, London, 1889.

[12] Grober, J. A., *Deutsch. Arch. klin. Med.*, 1901, lxix, 243.

The physiological properties of sulfocyanate and references thereto are summarized by Abderhalden.[13] The urine of healthy men contains on the average 0.0476 gm. of HSCN (0.0796 gm. of KSCN), somewhat less for women. According to Willanen[14] the administration to rabbits of glycocoll, creatinine, creatine, and adenine, which by oxidation or splitting yield HCN, causes increased urinary excretion of sulfocyanate. Lang[15] found that administration of acetonitrile, propionitrile, butyronitrile, capronitrile, and

TABLE V.

KSCN in Saliva and in 24 Hr. Urine at Admission and at Discharge.

Case No.	Saliva.		Urine.	
	Entrance.	Discharge.	Entrance.	Discharge.
	mg.	*mg.*	*mg.*	*mg.*
16	2.67	3.66	50.0	94.2
19*	0.69	1.25	60.9	67.3
21	1.90	2.13	51.8	68.6
23	1.07	1.47	26.1	76.4
24	0.89	2.24	15.7	58.2
26	1.61	2.56	52.9	55.1
28	1.80	1.08	78.2	47.9
29*	1.43	1.28	45.8	62.0
30	1.36	5.14	61.8	43.3
32	2.21	3.57	93.5	96.6
33	0.39	0.93	49.7	54.1
Average....	1.46	2.30	53.3	65.8

* The salivas at entrance for Cases 19 and 29 in this table were collected a day or two later than those of Table II—at the time the 24 hr. urine was being collected. So the sulfocyanate figures in the two tables do not agree for these two cases.

HCN increased urinary sulfocyanate. Gies and collaborators[6] in their study of sulfocyanate in relation to dental caries added much to the knowledge of the origin of sulfocyanate. They conclude from experiments with dogs that the addition to the diet of simple substances containing or yielding sulfide radicals do not markedly affect the sulfocyanate excretion. On the other hand, they

[13] Abderhalden, E., Biochemisches Handlexicon, Berlin, 1911, iv, 945.
[14] Willanen, K., *Biochem. Z.*, 1906, i, 129.
[15] Lang, S., *Arch. exp. Path. u. Pharmakol.*, 1894, xxxiv, 247.

found increased excretion of sulfocyanate after administration of acetonitrile (CH_3CN), and mandelic acid nitrile ($C_6H_5CHOHCN$), which contain the CN radical; and after administration of the amino-acids, alanine, glycocoll, and leucine, the first two of which Plimmer[16] found would by suitable oxidation yield hydrocyanic acid.

Sulfocyanate arises in the body from the union of $-SH$ and $-CN$ radicals both of which can be obtained from protein by various laboratory methods that involve chemical processes similar to those which obtain in the animal body. The production of $-SCN$ compounds appears to be a process of detoxification—a defensive measure similar to the detoxification of indole by the liver into indican normally found in the urine.

The analytical data given by Gies, Lieb, and Kahn on the sulfocyanate content of the tissues of dogs did not give positive support to the theory that sulfocyanate results from the metabolism of protein as such. They say

> "The production of sulfocyanate, as indicated by the excretion, was perceptibly decreased during fasting, but was increased very slightly if at all during overfeeding with protein. If, however, the production of sulfocyanate is estimated from the data for its distribution, the figures indicate that it was not materially affected by either fasting or overfeeding."

They suggest that sulfocyanate may result normally wholly from endogenous protein changes that are not materially affected by the amount of ingested protein. They regard the liver as the main organ of production of the sulfocyanate.

Dezani[17] has somewhat different views. Thus, in a series of articles, dealing with the genesis of thiocyanic acid in animals he declares Lang's hypothesis of a quantitative transformation of aliphatic nitriles into HCNS is inadmissible since six-sevenths to nine-tenths of the nitrile is decomposed in some other way. He did not find an increased urinary excretion of sulfocyanate on administering guanine, uric acid, glycine, aspartic acid, alanine, asparagine, or glycerol. In the dog, and in man as well as in rabbit, ingestion of cauliflower resulted in the elimination of

[16] Plimmer, R. H. A., *J. Physiol.*, 1904, xxxi, 65.

[17] Dezani, S., *Arch. farm. sper.*, 1917, xxiii, 245; 1917, xxiv, 113, 193; 1918, xxv, 83, 278; 1918, xxvi, 257; 1919, xxvii, 134; 1919, xxviii, 23.

HSCN in the urine. The thiocyanogenic substance belongs to the group of so called extractives. It is not a protein, or an amino-acid precipitable by phosphotungstic acid, or an organic sulfide. It does not occur in meat extract.

During a prolonged fast, a dog continued to eliminate HSCN in the urine to the extent of about one-third of that of a similar period preceding the fast. No relation was observed between the amount of total urinary nitrogen and of the HSCN eliminated. The elimination of urinary HSCN, however, depends on the nature of the food. In changing from a food poor in N to one rich in N the urinary HSCN excreted by the dog doubled in 24 hours. In the final paper of the series, Dezani concludes that the HSCN normally excreted by animals (guinea pig, rabbit, dog, man) is purely exogenous. Its origin is traced to the plant food consumed directly by herbivorous and indirectly by carnivorous animals.

That the excretion of sulfocyanate in the urine is somewhat independent of the total protein metabolized is shown by our urinary data in Table VI. Thus with low total urinary nitrogen in the tests made shortly after entrance to the hospital the nitrogen of the urinary KSCN is 0.1352 per cent of the total nitrogen while in the tests made shortly before discharge the nitrogen of the urinary KSCN is 0.0891 per cent of the total nitrogen. In short, though the sulfocyanate of the urine is, as a rule, increased at discharge from the hospital as compared with excretion at entrance, the increase is not in proportion to the increase of total nitrogen of the urine.

As pointed out by Gies the normal yield of sulfocyanate is, then, not dependent altogether on the total protein metabolized but on the production or evolution of CN from parts of protein; in short the production of sulfocyanate depends to a great extent on the nature of the protein and the amino-acid constituents thereof, and the capacity of the system to synthesize the sulfocyanate. As judged by the work of Willanen and of Gies, a protein yielding glycocoll, for example, should, barring lack of sulfur compounds in the food and barring insufficiency of the liver, give a greater yield of sulfocyanate in the urine.

A modifying factor in the urinary KSCN data obtained shortly after the patients' entrance to the hospital and again

TABLE VI.

Urinary KSCN.

Compared with total nitrogen, urea nitrogen, and ammonia nitrogen.

Case No.	Shortly after entrance.					Shortly before discharge.				
	Total N.	Urea N.	NH_3 N.	KSCN	KSCN (N) in percentage of total N.	Total N.	Urea N.	NH_3 N.	KSCN	KSCN (N) in percentage of total N.
	gm.	*gm. per cent of total N*	*gm. per cent of total N*	*mg.*		*gm.*	*gm. per cent of total N*	*gm. per cent of total N*	*mg.*	
16	10.595	8.798 83.0	0.532 5.0	50.0	0.0680	11.286	6.999 62.0	0.293 2.6	94.2	0.1203
15	8.604		0.265 3.1	34.1	0.0571	9.818	8.495 86.5	0.219 2.2	43.3	0.0636
19	8.659		0.321 3.7	60.9	0.1014	8.516	6.822 80.0	0.246 2.9	67.3	0.1139
20	5.854	3.089 52.8	0.922 15.8	79.6	0.1960	9.762	7.948 81.4	0.578 5.9	85.3	0.1260
21	5.538	4.150 74.9	0.254 4.6	51.8	0.1349	9.262	6.976 75.3	0.268 2.9	68.6	0.1068
23	1.700	1.009 59.3	0.113 6.6	26.1	0.2214	9.294	6.619 71.0	0.188 2.0	76.4	0.1185
26	5.673	4.341 76.5	0.236 4.2	52.9	0.1344	10.450	7.957 76.1	0.850 8.1	55.1	0.0760
28	7.430	5.560 74.8	0.205 2.8	78.2	0.1517	12.293	10.938 80.4	0.397 2.9	47.9	0.0520
29	6.579	2.992 45.5	1.181 17.8	45.8	0.1004	10.064	7.250 72.0	0.421 4.2	62.0	0.0888
30	4.760	3.667 74.9	0.270 5.7	61.8	0.1872	8.928	6.797 76.1	0.694 7.8	43.3	0.0699
31	5.098	2.198 43.1	0.738 14.5	52.2	0.1476	10.438	7.793 74.7	0.551 5.3	44.2	0.0611
32	9.906	5.957 60.1	1.673 16.9	93.5	0.1360	14.941	13.126 87.8	0.368 2.5	96.6	0.0932
33	5.885	4.240 72.1	0.365 6.2	49.7	0.1218	11.454	8.179 71.4	0.526 4.6	54.1	0.0681
Average ..	6.637	4.182 65.2	0.544 8.22	56.7	0.1352	10.577	8.146 76.5	0.431 4.1	64.5	0.0891

shortly before discharge lies in the change in diet. In the first tests the diet contained meat, which gives glycocoll on digestion; in the last tests milk was the main source of protein and milk contains little glycocoll. Despite the change in the diet in the two periods mentioned the absolute amount of sulfocyanate in the saliva and urine is, as a rule, greater during convalescence and shortly before the patients' discharge than during the active stage of the disease. The difference in favor of the convalescent stage would undoubtedly have been greater if the wider variety of diet used at entrance had been maintained throughout. As judged by work with a group of patients in 1917 the utilization of protein by pellagra patients tends to be somewhat subnormal.[18] The difference in the KSCN of the two periods must be due then to an increased assimilation and to an increase in detoxifying processes[19] or the sulfocyanogenetic powers of the organism as a whole.

In 1917 with marked cases of pellagra the difference in the sulfocyanate content of the saliva of the same patient in the active stage of the disease and in the convalescent stage was very marked. In 1919 with milder cases of pellagra the differences in the KSCN of saliva and of urine in the two periods were still noticeable.

The variations in sulfocyanate excretion between the active stage of the disease and the highly convalescent stage is associated with the difference in general condition. In the active stage of the disease when the patients are in a more or less cachetic condition the KSCN formation is less, as it is in other cachetic conditions as found by Grober. At discharge, when the patients have a greater feeling of well being and better assimilation, a higher protein metabolism, and presumably a greater detoxifying power of the system as a whole, the KSCN formation is increased. As the diet in the convalescent stage was if anything less ample than that of the active stage, the increase of sulfocyanate in the convalescent stage offers little support for the theory of its purely exogenous origin, but rather lends support to the view of Gies,

[18] Sullivan, M. X., Stanton, R. E., and Dawson, P. R., Metabolism in pellagra; a study of the urine, unpublished data.

[19] In the active pellagrous period one of us (S.) has found for several patients tested that the blood possessed a lower oxidative power as tested by the conversion of phenolphthalin to phenolphthalein.

Lieb, and Kahn that it is of endogenous origin. Thus the work herein outlined shows that the increased production of sulfocyanate is not closely bound up with total nitrogen but is more probably a product of some endogenous activity perhaps of the synthetic, detoxifying activity of the liver, coupled with a greater assimilation of sulfocyanogenetic complexes.

CONCLUSIONS.

The sulfocyanate content of the saliva and the urine of patients with active pellagra is, as a rule, less than that of the saliva and the urine of the same patients about to be discharged from the hospital as free from the signs and symptoms characteristic of pellagra.

The increase of the sulfocyanate of the saliva and urine seems to be associated with the betterment of the general condition of the patient, with better assimilation, a higher protein metabolism, and presumably a greater detoxifying power of the system as a whole.

The increase of sulfocyanate of the urine at discharge, over that of entrance, however, is not proportional to the increase of the total nitrogen of the urine.

I. THE CARBON DIOXIDE ABSORPTION CURVE AND CARBON DIOXIDE TENSION OF THE BLOOD OF NORMAL RESTING INDIVIDUALS.

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INTRODUCTION.

In 1919 Haggard and Henderson (1) outlined a method for the direct determination of the carbon dioxide tension of arterial and venous blood. They also demonstrated the applicability of the method to the study of abnormal physiological conditions involving disturbances of the respiratory system and the acid-base equilibrium in the blood. Stadie's (2) demonstration of the practicability of arterial puncture as a safe procedure suggested the application of Haggard and Henderson's method to the study of problems of human physiology and pathology. The method consisted of the simultaneous determination of the carbon dioxide absorption curve of the blood and the carbon dioxide content of the arterial and venous blood. By interpolating the arterial and venous values on the absorption curve the carbon dioxide tension and hydrogen ion concentration of the blood as they existed in the body were estimated. This method, with such modifications as were necessary to render it applicable to work with human subjects, has been employed by us in the study of a series of normal and pathological subjects.

In brief the procedure followed has been to obtain specimens of arterial and venous blood simultaneously, protecting them from contact with the air and consequent loss of CO_2 by means of albolene. Just before and just after the arterial puncture specimens of alveolar air were obtained by the Haldane and Priestley method (3). The carbon dioxide and oxygen content of both

arterial and venous specimens and the oxygen capacity of the blood were determined at once. Specimens of the blood (usually venous) were then exposed to various mixtures of CO_2 and air at 37.5°C. The carbon dioxide content of each specimen was measured and the absorption curve obtained from the results. The values for the CO_2 content of the arterial and venous blood and the CO_2 tension of the alveolar air were then placed on the curve by interpolation. In many cases we determined also the CO_2 capacity of the venous plasma.

This work was practically completed when Means (4) presented before The Association of American Physicians the results obtained by the application to fifteen normal and pathological subjects of a method for determining arterial carbon dioxide tension similar in all essential respects to our own. Means confined his efforts entirely to a determination of the absorption curve and the arterial CO_2 tension. To this we have added several other factors, especially the alveolar CO_2 tension. This has led us to draw different conclusions with regard to certain points. Dr. Means has kindly permitted us to see his paper in manuscript form so that we are able to call attention to these differences at this time.

Before this method could be applied to the study of pathological subjects it seemed necessary to ascertain the limits of variation of the various factors involved, in normal persons. As the subjects of this investigation three adult males were employed: J. P. (Case 1) and D. P. B. (Case 3), two of the authors of the paper; and W. S. M. (Case 2), Dr. William S. McCann, whose assistance in this respect and in other parts of the work we gratefully acknowledge. All three subjects had successfully passed examinations for the Medical Corps of the United States Army and on subsequent examinations had shown no signs of any existing pathological condition or abnormality that could in any way render their use as normal controls open to question. It will appear in the course of this investigation that some of our results fall definitely outside what has hitherto been considered as the range of normal variation. These deviations may be due to faults in the theory or methods employed by us or by previous observers. It may be that the amount of normal control material hitherto available has been too small to cover the entire range of

normal variation. It is possible that subsequent developments may prove that the deviations are indications in our subjects of pathological conditions at present undemonstrable by other methods.

The use of only three normal subjects offers an entirely inadequate amount of control material. The scope and nature of the investigation rendered it difficult to make a more extensive study. Of course it would have been possible to make use of selected patients in the hospital and thus enlarge the number of individuals in this group. The use of patients with pathological conditions as normal controls is, however, open to criticism in any investigation in which the influence of all factors attending these pathological conditions is not definitely established. The limits of variation of the carbon dioxide absorption curve as established by Straub and Meier (5) from a study of 56 hospital patients whose clinical condition, they say, gave no reason to suspect any pathological change of the blood, are very different from the limits of variation found by all other observers in known normal individuals.

If it were necessary to rely entirely on the results of our own observations for the delimitation of normal variations, certainly our material is inadequate. There is, however, a considerable mass of experimental data in the literature which we have employed freely for comparison and corroboration.

Details of Method as Applied to Both Normal and Pathological Subjects.

The experiments on patients were usually performed in the afternoon, about 2 hours after the midday meal. In some cases they were begun in the morning, 4 hours or more after breakfast. No attempt to establish standard dietary conditions was made. Most of the patients were unable to get out of bed. In the few cases in which the condition of the subject permitted him to be up and about, a preliminary rest period of 30 minutes was given. During this time the patient remained in the reclining position. The experiments on normal persons were usually performed very shortly after the midday meal and were preceded by a 30 minute rest period.

Specimens of alveolar air were obtained both before and after the arterial puncture. The arterial puncture was done under novocaine anesthesia. The blood was withdrawn under albolene into a Luer syringe and then placed under albolene in a test-tube from which specimens were removed for analysis within a few minutes.

The venous blood was collected as soon as possible after the arterial puncture. To prevent venous stasis no tourniquet was applied and the subject was directed to keep his arm relaxed. The blood was collected under albolene, without any contact with the air.

Coagulation of the blood was prevented by the addition of a minimal amount of dry, neutral potassium oxalate.

As soon as possible after the blood had been obtained from the subject under investigation, 3 cc. portions were placed in cylindrical separatory funnels of about 300 cc. capacity. These were filled with a mixture of CO_2 and air. The mixture was made up in a cylinder of about 1,200 cc. capacity and passed through the separatory funnel in an interrupted stream in order to wash the funnel out completely. Simultaneous analysis of the air in the cylinder and that in the separatory funnel showed differences of 0.2 per cent or less, indicating that the method was sufficiently accurate for our purposes. The mixture in the cylinder was analyzed for carbon dioxide each time. The distal opening of the separatory funnel was tightly stoppered while the gas was still flowing through it, and the pressure equalized by water level in the cylinder. The proximal stop-cock was now shut off and the separatory funnel rotated in a water bath at 37.5°C. for 20 minutes. As it was rotated with its long axis in the horizontal position a maximum blood surface was exposed to the gas mixture. At the end of the 20 minute period rotation was discontinued and the separatory funnel was placed in the vertical position long enough to allow the blood to collect in the narrowest portion. The neck was now withdrawn from the bath and carefully dried. The stopper was then removed and duplicate specimens were withdrawn by two observers as rapidly as possible in Ostwald pipettes calibrated to deliver 1 cc. between two points.

The carbon dioxide content of both specimens was determined simultaneously in two Van Slyke pipettes. The technique was the same as that described by Van Slyke (6) except for the fact that 20 per cent tartaric acid was substituted for N sulfuric. The coagulum produced by tartaric acid in whole blood is not so firm as that produced by sulfuric and consequently does not clog the pipette or render it difficult to clean. The blood was introduced into the cup of the pipette beneath a drop of dilute, carbon dioxide-free ammonia water to prevent the escape of CO_2. A blank determination of CO_2 in the reagents was made with each experiment and subtracted from the observed reading. This correction was rendered as small as possible by the use of carbon dioxide-free water and ammonia water. It was usually about 0.01 cc. On one or two occasions it rose to 0.02 or 0.025 cc.

Calculation and Extent of Error.

Owing to the fact that the gas mixture in the separatory funnel was equilibrated with atmospheric pressure at room temperature only, and not at 37.5°C., a correction had to be made for the altered CO_2 tension caused by the change in temperature, and for the vapor pressure. This should intro-

duce no error, as the concentration of carbon dioxide in solution is dependent directly on the carbon dioxide tension of the atmosphere with which it is in contact. The error that may occur from incomplete washing out of the separatory funnel is, as we have stated, less than 0.2 per cent, or 1.5 mm., and must lie always in the same direction. A further slight error is introduced by the loss or gain of CO_2 by the blood in coming to equilibrium with the air in the separatory funnel. This must be very small and can be more or less accurately estimated. The maximum amount of CO_2 change from this source was only 0.2 to 0.3 cc. per cc. of blood. With 3 cc. of blood in a 300 cc. separatory funnel this again might produce a change of 1.5 to 2.0 mm. in the CO_2 tension of the gas mixture. In point of fact this change must have been very much smaller in most instances. The error thus produced will vary in direction and extent according to the original carbon dioxide content of the blood. Roughly we may say that at carbon dioxide tensions below that of the blood employed the apparent CO_2 tension cannot be more than 1.5 mm. above or below the actual and most usually be much less, because the two main sources of error tend to compensate one another. At carbon dioxide tensions above that of the blood the apparent CO_2 tension may be as much as 3 mm. too high.

Duplicate determinations with the Van Slyke pipette should differ by not more than 1 volume per cent. Occasionally such agreement was not obtained. Sometimes this was directly referable to errors in technique; in some instances no such errors were discovered. The faulty agreement in these cases may have been occasioned by some loss of CO_2 due to a delay in the transfer of the blood from the separatory funnel to the pipette. On the whole we believe that this was a minimal source of error. The blood was withdrawn from the funnels with the least possible loss of time. Although we have made no direct experiments to determine the rate of loss of CO_2 under these conditions the fact that duplicate samples check so accurately in most instances indicates that such loss must be very slight.

A certain error is introduced by the volumetric measurement of small amounts of blood. The pipettes were carefully calibrated to deliver 1 cc. of distilled water between two marks with an accuracy of ± 0.002 cc. The error entailed in measuring a viscous solution like blood must vary with the physical characteristics of the individual specimen under investigation and cannot be estimated, but can never be entirely negligible. It must lie always in the same direction and tend to make the apparent volume of carbon dioxide too low.

Van Slyke (6) showed that the addition of potassium oxalate to plasma did not affect its carbon dioxide-combining capacity. De Corral (7) found that anticoagulant concentrations of potassium oxalate produced no recognizable effect on the hydrogen ion concentration of whole blood. In this case it should also be without effect on the carbon dioxide-combining capacity of whole blood. Joffe and Poulton (8), in point of fact, could demonstrate no change in the level of the absorption curve of fully oxygenated blood as a result of the addition of oxalate. This seems hardly possible, however, in view of the extreme lability and sensitiveness of the

ionic balance between cells and plasma. We have attempted to determine the presence and extent of the changes produced by the addition of potassium oxalate to whole blood.

Blood was drawn into a tube containing 1 mg. of hirudin for each 2.5 cc. of blood. 2 cc. portions of this blood were placed in two separatory funnels. To the blood in one of these were added 40 mg. of crystalline, neutral potassium oxalate. Both funnels were then filled with the same air-CO_2 mixture from a large spirometer and rotated at room temperature for 15 minutes. At the end of this time both specimens of blood were analyzed for CO_2 by the Van Slyke method. Other samples of blood were defibrinated and the effect of adding oxalate to defibrinated blood was also determined. The results appear in Table I.

TABLE I.

Effect of Potassium Oxalate in a Concentration of 2 Per Cent on the Carbon Dioxide-Combining Capacity of Blood.

	CO_2 content of blood after exposure to the same air-CO_2 mixture.		
	Without oxalate.	With oxalate.	Difference.
	cc.	*cc.*	*cc.*
Hirudinized blood.	0.685	0.686	+0.001
	0.696	0.734	+0.038
	0.696	0.728	+0.032
	0.559	0.620	+0.061
	0.597	0.695	+0.098
	0.569	0.600	+0.031
	0.554	0.618	+0.064
Defibrinated blood.	0.648	0.677	+0.029
	0.635	0.688	+0.053
Average difference.....			0.045

The addition of potassium oxalate in 2 per cent concentration produces an average increase of about 4 volumes per cent in the CO_2-combining capacity of the whole blood. This change occurs whether the oxalate is added to hirudinized or defibrinated blood. The increase was not constant in magnitude in all the observations. However, in this concentration oxalate produces visible physical changes in blood. The blood appears a lighter and brighter red. Whether this is due to hemolysis or not was not determined. The variations in our results may be somewhat dependent on the speed with which the oxalate was dissolved.

The concentration of oxalate employed in this experiment was far in excess of that used for the purposes of preventing coagulation. Such a concentration was chosen for the preliminary observations in order to

insure the detection of any change that might occur. When it was found that this change was constant in direction, the experiment was repeated with only 10 mg. of oxalate to each 2 cc. of blood (see Table II). In this concentration (0.5 per cent) which is well above that used to prevent coagulation, no visible physical changes in the blood were detected. The addition of oxalate increases the carbon dioxide-combining power of blood only about 1 volume per cent.

Christiansen, Douglas, and Haldane (9) found that if blood was allowed to stand its CO_2 capacity gradually diminished as if it became more acid. This change occurred more rapidly at body temperature than at the temperature of the room. For this reason we have exposed a fresh sample of blood to each gas mixture. In this way no sample remained at 37.5°C. more than 20 minutes. However, the multiplicity of procedures, involved

TABLE II.

Effect of Potassium Oxalate in a Concentration of 0.5 Per Cent on the Carbon Dioxide-Combining Capacity of Blood.

	CO_2 content of blood after exposure to the same air-CO_2 mixture.		
	Without oxalate.	With oxalate.	Difference.
	cc.	cc.	cc.
Defibrinated blood.	0.599	0.622	+0.023
	0.612	0.632	+0.020
	0.624	0.628	+0.004
	0.748	0.755	+0.007
	0.745	0.742	−0.003
	0.787	0.810	+0.023
	0.797	0.793	−0.004
	0.785	0.808	+0.023
Average difference			+0.0116

in simultaneous studies of so many factors, prolonged the total time of the experiments more than we should have wished. When only the absorption curve was determined, the duration of the procedure was within the limits of safety; in the complete experiments this was not always the case.

The order of procedure in the laboratory was:

I. The simultaneous determination of

1. The carbon dioxide content of arterial blood (in duplicate).
2. The carbon dioxide content of venous blood (in duplicate).
3. The oxygen content of arterial blood.

II. The determination of the oxygen content of venous blood. (In the later experiments four Van Slyke pipettes were used and the arterial and venous bloods were analyzed for oxygen at the same time, one observer manipulating both oxygen pipettes and extracting one specimen while the other was being laked.)

III. Determination of the oxygen capacity of the blood.

IV. Determination of the absorption curve. (Unfortunately the size of the water bath permitted the introduction of only one separatory funnel at a time.)

TABLE III.

Effect of Time on the CO_2-Combining Capacity of Blood.

	Time after withdrawal of blood.	CO_2 content of blood	Change of CO_2 content.
	min.	*cc.*	*cc.*
1. Hirudinized blood.	0	0.597	0.000
	120	0.569	−0.028
	165	0.554	−0.043
2. Defibrinated blood.	0	0.648	0.000
	60	0.635	−0.013
3. Defibrinated blood.	10	0.787	0.000
	65	0.797	+0.010
	170	0.785	−0.002
4. Oxalated blood.	30	0.720	0.000
	60	0.705	−0.015
	190	0.711	−0.009
	210	0.720	0.000
5. Oxalated blood.	5	0.790	0.000
	30	0.782	−0.008
	150	0.758	−0.032
	210	0.750	−0.040
6. Oxalated blood.	5	0.724	0.000
	60	0.723	−0.001
	125	0.703	−0.021
	180	0.676	−0.048
7. Defibrinated blood with 0.5 per cent oxalate.	10	0.810	0.000
	65	0.793	−0.013
	170	0.808	−0.002

In spite of the continuous work of three persons the total elapsed time of an experiment sometimes exceeded 3 hours. As skill and team work improved, this was steadily reduced until in the last observation we were able to complete the whole procedure within 2 hours from the time that the blood was withdrawn. It seemed advisable to find out the magnitude of the change which occurred in the blood on standing in the laboratory at

room temperature for this length of time. Blood was therefore withdrawn into oxalate and samples were removed at intervals, exposed to an air-CO_2 mixture, and the CO_2 content was measured. The results appear in Table III. Although not very consistent they indicate that the element of time may be a rather serious source of error. If blood is kept in an ordinary tube at room temperature, no change in the carbon dioxide-combining capacity can be demonstrated until the 2nd hour. At the end of 2½ hours a loss of about 2 per cent is found. After 3 hours this has increased to about 3 per cent. The fact that this loss occurred in some specimens, but not in others, suggests that it may be hastened by extraneous factors.

If all these errors should fall in the same direction one might conceivably obtain an absorption curve that was apparently 3.5 volumes per cent lower than the actual curve. The mean variation, however, is much smaller than this and cannot be considerably greater than the error of the actual measurement with the Van Slyke pipette. The general tendency is to make the curve low. The influence of these errors, in any case, cannot be very great as we are using the curves for the most part as a basis for comparative studies only.

Method of Representation.

In all charts the abscissa represents the carbon dioxide tension with which the blood is in equilibrium, expressed in mm. of mercury at 37.5°C., corrections having been made for the barometer and vapor tension. The ordinate represents the carbon dioxide content of whole blood, both physically dissolved and chemically combined. As the physically dissolved CO_2 is a direct linear function of the carbon dioxide tension, there is nothing to be gained by subtracting this from the total as is done by Haggard and Henderson (1).

For the calculation of the diagonals expressing pH, Henderson's formula has been used, modified to show the pH directly instead of his C_H7. The use of the negative logarithm, introduced by Sörensen, has become general and is convenient. The further multiplication of forms of expression can only tend to produce confusion and misunderstanding. The Henderson scale agrees fairly closely with that of Hasselbalch (10). That the calculation of the hydrogen ion concentration of the blood from the H_2CO_3: $B HCO_3$ ratio rests on a sound theoretical basis was first shown by L. J. Henderson (11). Hasselbalch (10) later demonstrated the accuracy of the method by practical experiments. Both Henderson and Hasselbalch dealt with completely oxygenated blood. Parsons (12) has recently questioned Hasselbalch's results on the ground that the application of the H_2CO_3: $B HCO_3$ ratio as a measure of hydrogen ion concentration is possible only in the case of homogeneous solutions such as plasma. Whole blood is a mixture consisting of two phases, cells and plasma. In a comparison of the hydrogen ion concentration measured electrometrically with that calculated by means of the H_2CO_3: $B HCO_3$ ratio in whole blood, Parsons found differences of as much as 0.09 in the pH. His values for pH are also

consistently higher than those of Hasselbalch. The difference of scale implied in the latter is of no importance if we use our figures only for comparative purposes. Until the point at issue is definitely settled it seems better to continue the use of the Hasselbalch pH scale with the realization that, if Parsons is correct, it should be rotated to the right 0.03 to 0.04 points. The discrepancy of 0.09 between calculated and observed values of pH found by Parsons indicates a considerable error in the method of calculation. This is, however, the maximum error. The average error, as we have calculated his results, is only about 0.03, if allowance is made for the difference in scale noted above. If we recognize the possibility of the presence of an error of this magnitude in our method of calculation we may use the values here obtained for purposes of comparison where the observed changes are large and consistent and occur in completely oxygenated blood. Work of Parsons (12) and L. J. Henderson (13) suggests that the application of the same scale to partially or completely reduced blood is not entirely proper because of the nature of hemoglobin. This will have little or no effect as regards the absorption curve figures, as these are determined on completely oxygenated blood. It may have considerable effect on the interpretation of the carbon dioxide content values of arterial and venous blood; but this will be discussed later. In any event each diagonal line must represent the locus of points on which the H_2CO_3:$BHCO_3$ ratio is identical.

The carbon dioxide capacity of the venous plasma is reduced to terms of alveolar carbon dioxide with which it should correspond if the normal relation found by Van Slyke obtained. The formula employed is

$$(\text{Mg. } CO_2 \text{ in chemical combination in 1 cc. plasma}) \times 35 = x$$

The Effect of Oxygen Unsaturation on the Carbon Dioxide Absorption Curve.

One difficulty at once presents itself. The absorption curve is obtained from completely oxygenated blood, while both the arterial and venous blood are partially reduced. Christiansen, Douglas, and Haldane (9), Hasselbalch (10), Parsons (12), and L. J. Henderson (13) have all shown that reduced blood has a greater power of combining with CO_2 than has oxygenated blood. All these observers employed defibrinated blood. Haggard and Henderson (14) reported recently that they had been unable to demonstrate this effect of oxygen on the absorption curve of oxalated blood. On the other hand Joffe and Poulton (8) found no difference in this respect between defibrinated and oxalated blood. The findings of the two observers are flatly contradictory. Collateral evidence, however, strongly favors the view of Joffe

and Poulton. The effect of oxygen unsaturation on the absorption of carbon dioxide in defibrinated blood is unquestionable. If oxygen unsaturation is without effect on the absorption curve of oxalated blood, the absorption curves of defibrinated and of oxalated venous blood should be quite different. Venous blood, as drawn, may be as much as 50 per cent unsaturated with oxygen. If this blood is defibrinated and then completely oxygenated, the usual procedure in the determination of the absorption curve, the change from partial reduction to complete oxygenation should make the absorption curve low in comparison with that of oxalated blood.

That this is not the case and that the carbon dioxide-combining capacity of oxalated blood is increased by oxygen unsaturation is shown by the following experiment.

50 cc. of blood were withdrawn from an arm vein of J. P. under albolene, without contact with the air. 25 cc. of this blood were defibrinated under albolene, while to the remainder was added a small amount of neutral potassium oxalate.

The oxygen content of both portions was then determined. The oxalated blood contained 7.1 volumes per cent of oxygen, representing an oxygen saturation of 33 per cent; the defibrinated portion contained 6.65 volumes per cent of oxygen, an oxygen saturation of 31 per cent.

Samples of each portion were then exposed for equal lengths of time to an air-CO_2 mixture of 38 mm. of CO_2 tension, at 37.5°C. Analysis at the end of this period gave 42.3 volumes per cent of CO_2 in the oxalated blood and 42.0 volumes per cent in the defibrinated. Both showed an oxygen saturation of 98.4 per cent.

Another sample of the oxalated blood was, at the same time, exposed to a mixture of CO_2 and nitrogen of 38 mm. of CO_2 tension. Analysis showed that this contained 47.5 volumes per cent of CO_2 and an oxygen saturation of only 21 per cent.

As a result of a reduction of 77.4 per cent in the oxygen saturation the carbon dioxide-combining power of the oxalated blood was increased 5.2 volumes per cent. This agreed with the value obtained by calculation from our empirical formula (see below), which was 5.66 volumes per cent.

That oxalated blood is suitable for the determination of the absorption curve is indicated by the experiments on the effect of oxalate detailed above (see "Methods"). Van Slyke and Cullen (15) have also shown that if arterial and venous bloods, oxalated, are exposed to the same mixture of CO_2 and air they are found to have the same carbon dioxide-combining power. If, then, oxygen does affect the carbon dioxide-combining power of blood in the

body, the addition of oxalate to the blood does not interfere with the reaction. As we shall point out later, unless such a reaction occurs in the body, certain respiratory phenomena become quite incomprehensible. At present the weight of evidence favors those who claim that oxygen influences the absorption of carbon dioxide in the blood. In this case arterial and venous carbon dioxide tensions must be corrected for oxygen unsaturation in order to approximate conditions that exist in the body.

On theoretical grounds Parsons (12) and L. J. Henderson (11) have concluded that the effect of oxygen on the CO_2-combining power of blood must depend on the amount of hemoglobin in the blood and the ratio of reduced hemoglobin to oxyhemoglobin. Christiansen, Douglas, and Haldane (9) and Joffe and Poulton (8) have presented experimental data that conform to this theory. It should be possible, then, to find an empirical mathematical expression for the effect of oxygen unsaturation on the level of the carbon dioxide absorption curve, the accuracy of which will depend on the accuracy of the data at our disposal. The simplest equation that will express the fact that the effect of oxygen unsaturation on the curve is dependent on the amount of reduced hemoglobin is

$$K \times \mathrm{Hb} = D$$

where K = a constant
Hb = the oxygen unsaturation of the blood expressed in volume per cent of oxygen
D = the change of level of the absorption curve as a result of the effect of oxygen unsaturation, expressed in volume per cent of carbon dioxide

Of course this formula cannot express the reaction accurately. K cannot be a simple constant because the curves of reduced and oxygenated blood meet at the origin. K must, therefore, have a value of zero at 0 mm. of CO_2 tension and must increase as the CO_2 tension rises. The value of K may, however, be sufficiently constant at CO_2 tensions that exist in the body to permit the use of such a simple equation.

As experimental data we have the carbon dioxide absorption curves of the blood of J. S. H. (Christiansen, Douglas, and Haldane) saturated with oxygen and 98 per cent reduced and the curves of the blood of J. (Joffe and Poulton) completely oxy-

genated and 90 per cent reduced. The hemoglobin values given for J. S. H. and J. are 100 and 101 per cent respectively, according to the Haldane scale. To these data we have applied the above equation and calculated values of K. The results are

TABLE IV.

Effect of Oxygen on the Carbon Dioxide Absorption Curve of Whole Blood, from Equation $K \times Hb = D$.

Subject.	CO_2 tension.	Observed value of D.	Value of K.
	mm. Hg		
J. S. H. (reduced blood 2 per cent saturated with oxygen. Hemoglobin 100 per cent.)	0	0	0
	10	5.5	0.303
	20	5.5	0.303
	30	5.6	0.309
	40	6.0	0.331
	50	6.2	0.342
	60	6.2	0.342
	70	6.6	0.364
	80	6.7	0.370
	90	7.0	0.368
J. (reduced blood 10 per cent saturated with oxygen. Hemoglobin 101 per cent.)	0	0	0
	10	4.5	0.268
	20	5.3	0.315
	30	5.6	0.333
	40	5.5	0.327
	50	5.5	0.327
	60	5.9	0.351
	70	6.5	0.386
	80	7.4	0.440
	90	8.0	0.476
Average values from 30 to 70 mm. inclusive.....		J. S. H.	0.337
		J.	0.345

shown in Table IV. It will be seen that the average values of K between 30 and 70 mm. of CO_2 tension are practically identical, averaging 0.34, and that the maximum deviation from this mean at these tensions is only about 10 per cent. This is within the

error of the method employed and a constant value of K can therefore be assumed for our purposes. We have applied this equation to our results in order to correct the arterial and venous CO_2 tensions observed for the effect of oxygen unsaturation. In practice a curve is drawn at the distance D above the curve of completely oxygenated blood and the corrected point is placed where the carbon dioxide content of the blood intersects this curve.

In the charts of experiments and in the tables of this paper the arterial and venous points are shown both in relation to the absorption curve of completely oxygenated blood and also after corrections have been made for oxygen unsaturation. The quantitation of the effect of oxygen unsaturation is, of course, based on insufficient data. If, however, the data of Haldane and of Joffe are correct and the value of K does vary by only 10 per cent, the maximum error in such a correction cannot be more than one-tenth of the variation produced by the change from the completely oxygenated to the completely reduced state. For a hemoglobin of 100 per cent the error would be 0.64 volume per cent, well within the limit of error of the analytical methods we have employed. Even a considerable error in the equation would make no distinguishable difference in the results.

Level of the Absorption Curve of Normal Resting Subjects.

Christiansen, Douglas, and Haldane (9), in 1914, studied the carbon dioxide absorption curve of two normal individuals (J. S. H. and C. G. D.) at rest, and during and after exercise. They also made a few observations on five other normal persons at rest. At about the same time Morawitz and Walker (16) made some observations on the carbon dioxide-combining power of whole blood, by an entirely different technique, and obtained almost identical results. Hasselbalch (10), in 1917, determined the curve of the normal subject, K. A. H. In 1918 Straub and Meier (5) published the results of a study of the absorption curves of 56 hospital patients in whom, they state, there was no reason to suspect any pathological change of the blood. Liljestrand and Lindhard (17), Krogh and Liljestrand (18), Joffe and Poulton (8), Parsons (12), Davies, Haldane, and Kennaway (19),

and Means, Bock, and Woodwell (4) have also given normal absorption curves.

In Fig. A are presented the limits of variation of the carbon dioxide absorption curves of normal resting individuals which we have been able to find in the literature, and those of three normal persons which we have determined. Some of these represent only single observations. Others are the results of repeated studies. Altogether observations on twenty-one normal persons appear, besides Straub's 56 hospital patients.

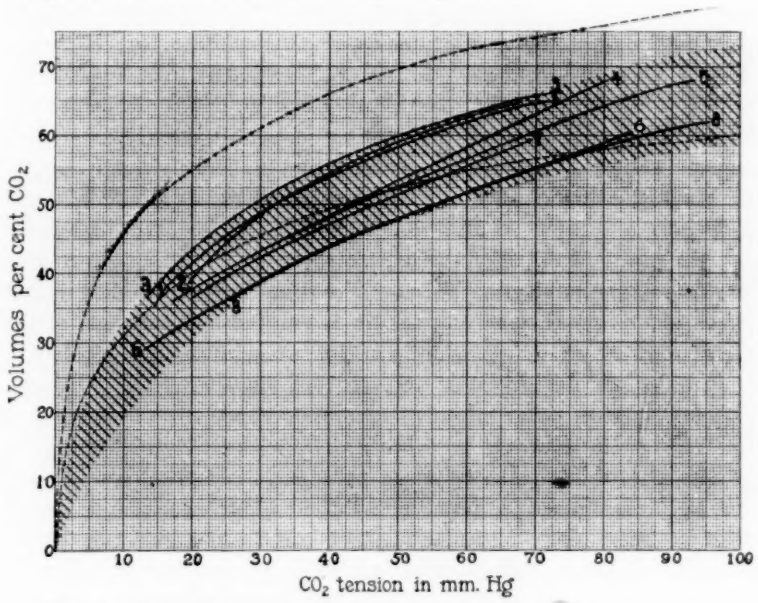

FIG. A. Absorption curves of three normal subjects.

1. J. P., Feb. 27, 1920.
2. " Mar. 5, 1920.
3. " " 12, 1920.
4. W. S. M., Feb. 13, 1920.
5. " May 21, 1920.
6. D. P. B., Mar. 26, 1920.
7. " " 29, 1920.
8. " May 14, 1920.

Shaded area shows the range of variation of the absorption curves of seventeen normal individuals, obtained from the literature. The area enclosed by broken lines represents the range of variation exhibited by Straub and Meier's 56 cases.

The methods employed are almost as numerous as the authors. Nevertheless, with the exception of Straub's highest figures, the agreement in the results of different observers is remarkably close. The normality of Straub's cases may reasonably be questioned and the data which he presents are not sufficient to permit analysis of his results. The simple statement that the clinical condition of these patients gave no reason to suspect any pathological change of the blood is not a sufficient characterization to warrant the assumption that they are normal subjects. As yet the study of the carbon dioxide absorption curve of whole blood has been too limited to permit one to say that pathological changes occur in certain restricted conditions, only. It seems better, then, to omit Straub's figures in discussing the limits of variation of the absorption curve in normal subjects. If this is done, it will be seen that the limits of variation of the eighteen cases reported by other observers are the same as those found in our three normal subjects. For the present, these must be considered the limits of range of the carbon dioxide absorption curve of normal resting subjects. It may be that further studies will reveal greater variations or that more careful investigation will prove that the extreme curves are due to some pathological or abnormal physiological condition not yet discovered.

The carbon dioxide content of blood is composed of two parts: (1) That portion which is present in simple solution and as H_2CO_3; (2) that portion which is present in chemical combination as bicarbonate. The amount of CO_2 in solution at any given temperature is dependent entirely on the tension of carbon dioxide to which the blood has been exposed, and can be calculated directly from this and the solubility coefficient. It has been shown by Bohr (20), L. J. Henderson (11), and more recently by Parsons (12) that practically all the chemically combined CO_2 must exist as bicarbonates. Henderson has also shown that the concentration of bicarbonates may be used as a measure of the alkali of the blood not bound by acids other than carbonic, without any significant error. If this is so, the alkali reserve of the blood must determine the height of the absorption curve.

Inspection of the curves discloses the fact that they vary considerably in shape and slope. In most cases these variations are minimal within the limits of CO_2 tension that are believed to

obtain in the arterial blood. If the curves of patients with severe anemia are omitted, the remainder will be found, with few exceptions, to maintain the same relative height within the limits 30 to 60 mm. In order to compare the heights of different absorption curves, it is necessary to study them at the same level. The ideal level from a theoretical standpoint is the one that conforms as nearly as possible to the conditions supposed to exist in the body; that is, where the absorption curve crosses the pH 7.35 line. In the lowest curves, however, this point lies at an extremely low carbon dioxide tension, occasionally below those tensions employed in the experimental work. The use of the point of intersection with the pH 7.35 line would, in these instances, demand extrapolation with the introduction of a considerable error. We have, therefore, chosen the point at which the absorption curve crosses the 40 mm. line. This point has been chosen because it is the generally accepted figure for average, normal alveolar CO_2 tension and because it has been rather generally employed by other investigators in the determination of the CO_2 capacity of whole blood and plasma. Except in certain pathological conditions which we shall discuss later, the differences in the results of the two methods (estimation of height at the 40 mm. line and at the pH 7.35 line) are insignificant. The height of the curve at either of these points may be considered to represent the alkali of the blood available for combination with CO_2 under conditions that exist in the body. This is expressed in terms of total carbon dioxide instead of carbon dioxide present as bicarbonate. The latter may be derived by subtracting 2.7 volumes per cent from the value given. The results obtained by others and by us, from both normal and pathological subjects, appear in Table V.

From these figures the height of the normal absorption curve at 40 mm. of CO_2 tension may be said to lie between 43 and 56 volumes per cent, with an average of about 50. The limits of the whole number of normal curves are practically the same as those found in our three normal subjects, if Straub's figures are omitted.

Christiansen, Douglas, and Haldane (9) found that, although the absorption curves of different individuals differed considerably in level, the absorption curve of any given individual was constant and quite characteristic in both height and shape.

TABLE V.

Height of the Absorption Curve at 40 Mm. CO_2 Tension.

Subject and condition.	CO_2
	vol. per cent
Christiansen, Douglas, and Haldane: 6 normal subjects; limits	46.6
of variation	51.2
Morawitz and Walker: 1 normal subject; limits of variation	51.7
	54.2
Hasselbalch: 1 normal subject, K. A. H	50.0
Krogh, Liljestrand, and Lindhard: 5 normal subjects; limits of	44.0
variation	48.3
Joffe and Poulton: 1 normal subject, J	45.1
Parsons: 1 normal subject, T. R. P., Oct., 1917	51.9
July, 1918	46.7
Davies, Haldane, and Kennaway: 1 normal subject, J. B. S. H.	49.4
Means, Bock, and Woodwell: 2 normal subjects, M. N. W	51.1
J. H. M	50.9
Straub and Meier:* 56 hospital cases with presumably normal	49.5
blood; limits of variation	65.6
J. P., normal subject, Feb. 27, 1920	53.9
Mar. 5, 1920	54.5
" 12, 1920	55.9
W. S. M., normal subject, Feb. 13, 1920	48.3
May 21, 1920	48.2
D. P. B., normal subject, Mar. 26, 1920	43.3
" 29, 1920	47.0
May 14, 1920	43.6

Summary.

	No. of cases.	CO_2 at 40 mm.		
		Maximum.	Minimum.	Average.
		vol. per cent	*vol. per cent*	*vol. per cent*
Other observers*	18	54.2	44.0	49.3
Subjects of this series	3	55.9	43.3	49.2
All normal subjects	21	55.9	43.3	49.3

* Straub and Meier's figures are omitted from this summary for reasons given in the text.

Our results seem to confirm this statement. The total variation in the height of the curve of J. P. (Case 1), determined three times in a period of 6 weeks, was only 2 volumes per cent. Two curves of W. S. M. (Case 2), obtained over 3 months apart, show even closer agreement. In the case of D. P. B. (Case 3), two curves at an interval of 6 weeks are almost identical. A third, taken 3 days after the first, lies about 4 volumes per cent higher. Christiansen, Douglas, and Haldane showed that exercise reduced the level of the curve. The variations that were found may have been due to a failure to establish proper resting metabolic conditions. The two curves of Case 3 that agree most exactly were obtained under standard conditions. The very close agreement of these curves derived from a considerable number of observations, scattered over a period of 2 months or more, can hardly be a matter of coincidence. It is also additional evidence that, for purposes of comparative study at least, the errors of the method are of no great importance.

The meaning of the differences in the height of the absorption curves of different individuals is not clear. That it is paralleled by no corresponding difference in the carbon dioxide tension of the alveolar air has been stated by Christiansen, Douglas, and Haldane. By a strange chance it happens that the absorption curves of Cases 1 and 3 are very different and lie respectively at the extreme upper and lower limits of the normal range. Both persons have been studied by a variety of respiratory methods and they have been found to differ in many respects. Some of these differences and their possible bearing on the height of the absorption curve will be pointed out later.

Carbon Dioxide Tension and the Hydrogen Ion Concentration of the Arterial Blood of Three Normal Subjects.

The carbon dioxide tension of the arterial blood of three normal persons (see Table VI, Columns 4 and 5; and Charts 1, 2, and 3[1]) was found to be 36 to 50.5 mm. of Hg uncorrected for oxygen unsaturation, 34.5 to 49 mm. after correction. This variation

[1] To facilitate the reference to charts and tables, the same numbering has been retained throughout tables, charts, and protocols. Thus Chart 1 refers to Case 1. Charts of general characteristics are lettered.

TABLE VI.

Results of the Study of Three Normal Subjects.

Subject.	Date.	Alveolar CO_2 tension.	Alveolar reaction.	Arterial CO_2 content.	Arterial CO_2 tension.		Arterial reaction.		Venous CO_2 content.	Venous CO_2 tension.		Venous reaction.		Difference between arterial and alveolar CO_2 tension.	Difference between venous and arterial CO_2 content.	Difference between venous and arterial CO_2 tension.	Difference between venous and arterial reaction.
					Uncorrected.	Corrected.	Uncorrected.	Corrected.		Uncorrected.	Corrected.	Uncorrected.	Corrected.				
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
	1920	*mm. Hg*	*pH*	*vol. per cent*	*mm. Hg*	*mm. Hg*	*pH*	*pH*	*vol. per cent*	*mm Hg*	*mm. Hg*	*pH*	*pH*	*mm. Hg*	*vol. per cent*	*mm. Hg*	*pH*
1. J. P.	Feb. 27								57.4	47.5		7.33					
	Mar. 5								64.3	65.5		7.23					
	" 12	38.7	7.42	54.1	36.0	34.5	7.43	7.45	62.0	56.3		7.28		−4.2	7.9		
	May 19	36.0	7.42	54.1	39.0	38.7	7.40	7.40	55.6	42.0	39.5	7.37	7.40	2.7	1.5	0.8	0.00
2. W. S. M.	Feb. 13								64.1	72.0		7.19					
	May 21	35.9	7.35	50.9	47.0	45.0	7.28	7.30	59.4	67.5	52.5	7.18	7.30	9.1	8.5	7.5	0.00
3. D. P. B.	Mar. 26	38.5	7.29	45.5	45.0		7.25		53.4	65.0		7.15			7.9		
	" 29	37.6	7.33						57.5	65.0		7.18					
	May 14	37.4	7.30	47.9	50.5	49.0	7.22	7.23	56.2	72.0	58.5	7.12	7.22	11.6	8.3	9.5	0.01
Maximum		38.7	7.42	54.1	50.5	49.0	7.43	7.45	64.3	72.0	58.5	7.37	7.40	11.6	8.5	9.5	0.01
Minimum		35.9	7.29	45.5	36.0	34.5	7.22	7.23	53.4	42.0	39.5	7.12	7.22	(0.0)	1.5	0.8	0.00

did not bear a direct relation to the height of the corresponding absorption curves. The consequence is that our results indicate a considerable range of variation in the pH of normal arterial blood (see Table VI, Columns 6 and 7); from 7.22 to 7.43 uncorrected for oxygen unsaturation, 7.23 to 7.45 after correction. This is somewhat larger than the variation found by Michaelis (21) by direct electrometric titration in the venous blood of twelve normal resting persons, which was 7.28 to 7.43. As we shall show later the pH of venous blood is practically the same as that of arterial blood so that Michaelis' figures and ours are quite comparable. His average pH is 7.35, ours is 7.32, a very fair agreement. There are, as yet, no direct determinations of the pH of human arterial blood to be found in the literature. Most observers have relied, instead, on the values obtained from venous blood equilibrated with alveolar air.

Parsons' (12) objections to the use of the ratio of free to combined carbon dioxide as a measure of the hydrogen ion concentration of whole blood have been discussed above. If these objections are valid our figures cannot be interpreted as absolute values, but they must be very close to the true values. The differences of pH in the three individuals, moreover, are too great to be accounted for by errors in the method of calculation. Parsons considers the maximum error in calculation to be about 0.09; Hasselbalch (10) found the maximum error no greater than that of the method for determining CO_2, which he places at a difference of 0.04 in the pH. This may be increased somewhat by the error in the oxygen correction formula. A greater error, however, results if this correction is not made. Certainly it expresses the change in pH as in carbon dioxide qualitatively and, as we have shown, deals with a value of such small magnitude that the error must be minimal, probably not greater than 0.02 in pH. On the whole the maximum error should never exceed 0.06 and the average error must be nearer 0.02 to 0.03.

It is quite difficult to believe that the differences in pH found in J. P. (Case 1) and D. P. B. (Case 3) are due to errors in method, because repeated determinations revealed the same differences. Values of 7.22 and 7.25 were found in Case 3 at an interval of 6 weeks. In Case 1 in two experiments more than 2 months apart, arterial CO_2 contents were found to be identical. Unfortunately

on the second occasion the determination of the absorption curve failed. However, Case 1's absorption curve had been sufficiently well established by three other observations to warrant the assumption that the arterial CO_2 tension and the hydrogen ion concentration were the same on both occasions. In order to represent this second experiment graphically (see Chart 1) we have employed an absorption curve made up from the three previous experiments. Under these conditions the arterial pH of Case 1 on these two occasions was 7.40 and 7.45.

There is a variation in the pH of normal resting individuals of about 0.2, a little more than has been generally assumed. There seems to be a tendency for each individual to maintain his pH at a constant level. This is characteristic of the individual. We have other evidence that this must be so in the fact that the level of the carbon dioxide absorption curve and the level of the alveolar CO_2 tension, both determinants of the hydrogen ion concentration of the blood, are both constant in any given individual, although subject to a certain variation in different individuals.

If this is so one cannot talk in absolute terms of compensated or uncompensated acidosis or carbon dioxide retention from a single observation of the arterial pH under pathological or abnormal physiological conditions. If Case 1 normally maintains a pH above 7.4 in his arterial blood a pH of 7.30 would, in his case, indicate a retention of carbon dioxide or an uncompensated acidosis. But the same pH (7.30) would be normal for Case 2 and would mean an uncompensated alkalosis in the blood of Case 3. It is necessary to establish the normal pH level of an individual before one can accept as proof of uncompensated acidosis or alkalosis any values except such as lie beyond the limits of normal variation.

Relation of Alveolar and Arterial Carbon Dioxide Tension.

Hasselbalch (10) has shown that if the blood of an individual is exposed at body temperature to a mixture of CO_2 and air of the same CO_2 tension as that of the alveolar air of the subject, the pH of the blood will be found to vary only slightly about a mean of 7.35. This must mean that, in general, the alveolar carbon dioxide tension varies with the height of the absorption curve.

On the other hand, Christiansen, Douglas, and Haldane (9) make the statement that the alveolar CO_2 tension does not vary with the height of the absorption curve.

Table VII gives the calculated pH of blood equilibrated with an air-CO_2 mixture of the same CO_2 tension as that of the alveolar

TABLE VII.

pH of Blood Exposed to an Air-CO_2 Mixture of the Same CO_2 Tension as That of the Alveolar Air of the Individual from Whom Blood Was Removed.

(Values obtained by calculation from the absorption curve.)

Observer.	Subject.	Alveolar CO_2 tension.	Reaction.
		mm. Hg	*pH*
Christiansen, Douglas, and Haldane.	J. S. H.	40.8	7.35
	C. G. D.	38.5	7.35
	J. C.	35.0	7.35
	J. G. P.	40.2	7.35
Krogh and Lindhard.	A. K.		7.34
	J. L.		7.30
Means, Bock, and Woodwell.	J. H. M.	40.6	7.37
	M. N. W.	37.5	7.35
	1920		
The authors.	J. P.		
	Mar. 12	38.7	7.42
	May 19	36.0	7.42
	W. S. M.		
	May 21	35.9	7.35
	D. P. B.		
	Mar. 26	38.5	7.29
	" 29	37.6	7.33
	May 14	37.4	7.30
Average			7.35
Maximum			7.42
Minimum			7.29

air in the three normal persons of our series; four normal persons given by Christiansen, Douglas, and Haldane; A. K. and J. L. from data presented by Krogh and Liljestrand (18) and Krogh and Lindhard (22); and J. H. M. and M. N. W. from Means, Bock, and Woodwell (4). In the case of the subjects A. K. and

J. L. the absorption curves were taken from recently published work, while the figures for alveolar CO_2 tension employed were calculated from several series of observations reported in earlier studies. In all the other cases including our own, absorption curves and alveolar carbon dioxide were determined at the same time. We have calculated the pH by taking the point where the absorption curve of the individual crosses the line representing his alveolar CO_2 tension.

The average normal pH under these conditions is, as Hasselbalch found, 7.35. In spite of the statement of Christiansen, Douglas, and Haldane that they could find no exact relation between the height of the absorption curve and the alveolar carbon dioxide tension, their figures show a remarkable agreement. The difference between our conclusions and those of Christiansen, Douglas, and Haldane may be partially explained by unavoidable differences in the construction of the curves. Hasselbalch's calculations of the "alveolar pH" of J. S. H., C. G. D., and K. A. H. differ from ours by 0.01 and 0.02. This is about the error that should be expected in the construction of the most probable curve from a series of points that has an inherent error as great as that of methods for determining carbon dioxide in blood. The failure of Christiansen, Douglas, and Haldane to recognize the relation between the alveolar carbon dioxide tension and the height of the absorption curve may have been due also to the fact that this relation is not a linear function. They say that although the alveolar CO_2 of J. C. is 5 to 6 mm. lower than that of J. S. H. the corresponding absorption curves are almost indistinguishable. As we have reconstructed the curves from their data, that of J. C. appears to be 2.5 to 3.0 mm. lower than that of J. S. H. Owing to the slope of the pH lines and the dissociation curves, the difference in CO_2 tension between the points of intersection of these curves with the pH 7.35 line is about 6 mm.

The results of our experiments are not quite so satisfactory. Case 1 gives a pH of 7.42, while Case 3's is 7.29 to 7.33, with an average of 7.30. The fact that repeated observations gave identical results suggests that these relations are individual characteristics of the subjects under investigation. If we can accept the composite data for J. L. it is interesting to note that both his

alveolar CO_2 tension and absorption curve agree with those of Case 3, giving in both cases a low pH of only 7.30. In both, the absorption curves are so low that an alveolar CO_2 tension of 30 to 31 mm. would be required to produce a pH of 7.35. This is below the figures usually accepted for the lower limits of the normal range of alveolar CO_2 tension. Arguing in the same way, Case 1 would require an alveolar CO_2 tension of 45 mm. to bring his pH to the 7.35 line. Such an alveolar tension has never been observed in this subject although he has been studied for 5 years.

When the alveolar CO_2 tension is compared with that of the arterial blood (see Table VI, Column 12), the close agreement which has been generally assumed does not appear. From Charts 1, 2, and 3 it is found that, in Case 1, Experiment 1, the alveolar CO_2 tension is 4 mm. higher than that of the arterial blood; in the other two cases the alveolar tension is 6 to 11 mm. lower than that of the arterial blood.

The difference found in Case 1 can hardly be accepted at its face value. One might consider the findings in this case an indication that Krogh (22) and his followers were right in their statement that the values obtained by the Haldane method were too high. But every other observation here reported contradicts this. It is not easy to believe that there was an error in the collection or analysis of the alveolar samples. The subject is experienced in respiratory methods. Duplicate specimens checked perfectly. Not only is this true in this experiment, but in the experiment 2 months later values obtained for both alveolar CO_2 tension and arterial CO_2 content were almost identical with those of the earlier experiment. Finally the alveolar CO_2 was determined in conjunction with a respiratory experiment in which the dead space was calculated by the Haldane (3) formula. With the alveolar CO_2 as determined, a dead space of 130 cc. was obtained. If the alveolar CO_2 tension were assumed to be only 1.5 mm. below the arterial tension, the dead space would have to be as small as 75 cc. and the alveolar tension only 33 mm. Determinations of the dead space by Pearce's (23) method on the same subject gave a value of 130 cc. and his alveolar carbon dioxide tension has never been found as low as 35 mm. An alveolar CO_2 tension of 33 mm. would also, if placed on the dissociation

curve, give a pH of 7.47. The fault is, therefore, probably not in the determination of the alveolar CO_2 tension.

If the absorption curve were only 1.5 volumes per cent lower, the alveolar and arterial tensions would coincide. As we have pointed out, there is sufficient error in the method of determining the absorption curve to make it impossible to draw any conclusions from such small differences. The curve obtained on March 12 is perceptibly higher than three others obtained from the blood of Case 1 on other dates. If the average of all the curves of Case 1 is employed it is found that, in the experiments of March 12 and May 19, the alveolar CO_2 tension is respectively the same as and 3 mm. lower than the arterial tension. In Chart 1 such an average absorption curve has been used (Curve 2) in representing the experiment of May 19. For the experiment of March 12 (Curve 1), the absorption curve as actually determined on that day has been used. Curve 2, we believe, probably represents more nearly the actual relation between alveolar and arterial CO_2 tension in both experiments. In this case the two are, for practical purposes, identical.

The remaining cases are, however, not at all in keeping with the accepted theory which regards the alveolar CO_2 tension as a measure of the arterial tension. Krogh and Krogh (24) found that the alveolar CO_2 tension of rabbits was about 0.1 to 0.2 per cent lower than that of the arterial blood. Our experiments show a difference of 0.8 to 1.5 per cent in normal persons and, as we shall show, a much greater difference in some pathological conditions. Against the assumption that the alveolar CO_2 is in error the same arguments which were proposed in Case 1 may be advanced. In order to make the alveolar and arterial tensions agree by correcting the absorption curve the latter would have to be placed at least 2.5 volumes per cent higher. But in both these subjects repeated determinations of the absorption curve agreed perfectly. It is improbable that a systematic error would occur in four determinations on two subjects and fail altogether to appear in three experiments on a third. The difference between arterial and alveolar CO_2 tension in these cases seems to be a real one.

CO_2 Tension and Hydrogen Ion Concentration of Venous Blood and the Difference between Arterial and Venous Blood.

Results obtained from the analysis of venous blood are inherently less reliable than those obtained from arterial blood because the carbon dioxide and oxygen tension of the venous blood are subject to the influence of local conditions. The influence of venous stasis on both CO_2 content and CO_2-combining capacity has been brought out by Morawitz and Walker (16), as has the effect of exercise. Although it is possible to avoid artificial stasis by omitting the use of a tourniquet, it is impossible to control completely local changes in the blood flow or in the muscular activity of the arm. The fact that no simultaneous studies of arterial and venous blood from either normal or pathological subjects have given consistently satisfactory respiratory quotients is an indication that such factors are active even under the most carefully controlled conditions. Harrop's (25) blood respiratory quotients range from 1.81 to 0.62 in normal persons, 1.76 to 0.25 in cardiac subjects. Our own vary from 1.62 to 0.34. Arterial blood is not subject to the influence of local circulatory conditions and it is, therefore, probable that the chief source of error lies in the venous blood. It is obvious that, unless satisfactory quotients are obtained, the results cannot be considered to represent conditions that exist in the general circulation. Nor is it proper to accept as satisfactory isolated experiments in which satisfactory quotients are obtained. The method must be capable of returning such quotients with a certain degree of regularity in order to permit its use for the determination of the blood flow, venous CO_2 tension, and other similar characteristics in individual experiments. It may be possible, however, to draw general conclusions from the study of a considerable number of experiments.

In Table VI, Columns 9, 10, 11, 12, and 15, and Charts 1, 2, and 3 are given the CO_2 tension and the hydrogen ion concentration of the venous blood of the three normal subjects of our series with the difference in CO_2 tension between the arterial and venous blood. The CO_2 tension of venous blood was found to vary between 42 and 72 mm. uncorrected for oxygen unsaturation, 39.5 to 58.5 mm. after correction, with an average of 50.2

mm. The corresponding values for pH were 7.37 to 7.12 uncorrected, 7.40 to 7.22 corrected, with an average of 7.31. The difference between arterial and venous CO_2 tension was from 0.8 to 9.5 mm. corrected.

The range of variation of all these factors is very considerable. Especially noticeable is the very high venous carbon dioxide tension found in Cases 2 and 3. The first attempt to ascertain the venous carbon dioxide tension was made by Christiansen, Douglas, and Haldane (9) in 1914. Since then many other workers have proposed methods. All these methods are indirect and all depend on the same principle, the use of the lungs as a tonometer. The subject rebreathes a certain amount of air or some other gas mixture for a length of time less than that consumed by a single complete circulation of the blood. During this time the rebreathed air is supposed to attain equilibrium with the venous blood in the pulmonary circulation. From the CO_2 tension of the rebreathed mixture the CO_2 tension of the venous blood is calculated after a correction has been made for oxygen unsaturation. In order to find the arterial CO_2 tension the method of Haldane has been most commonly employed. By the use of these or similar methods all observers have found a difference between the arterial and venous carbon dioxide tension of from 4 to 7 mm. In some studies on the subjects J. P. (Case 1) and D. P. B. (Case 3) made with the Haldane method for arterial CO_2 tension and the Henderson (26) method for venous CO_2 tension we found the difference between the two to be from 2.7 to 7.0 mm. uncorrected for oxygen unsaturation, 1.6 to 4.2 mm. after correction. (The method of correction proposed by Christiansen, Douglas, and Haldane was used. In this 40 per cent of the total difference in CO_2 tension is subtracted as the effect of oxygen unsaturation.) The results obtained by our direct method and those obtained by the older, indirect methods are entirely incompatible. The new method gives more variable, but on the whole higher values.

It may be objected that it is not proper to criticize a method that aims to measure the carbon dioxide tension of the mixed venous blood in the pulmonary circulation on the basis of data obtained from the analysis of venous blood taken from an extremity. It may well be questioned whether the oxygen unsaturation

of the mixed venous blood of Cases 2 and 3 was as great as that found in the arm veins. The oxygen unsaturation in Case 3, Experiment 2, was 67.8 per cent and that of Case 2 was 76.7 per cent. (Lundsgaard (27), Stadie (2), and Harrop (25) found no normal subjects with a venous oxygen unsaturation of more than 40 per cent.) But this cannot explain the whole difference between the results of the direct and indirect methods. The marked oxygen unsaturation will be at least partially compensated by a change in the carbon dioxide-combining capacity of

TABLE VIII.

Difference between Arterial and Venous CO_2 Tension.

(Calculated from ten normal cases presented by Harrop.)

No.	Arterial CO_2 content.	Arterial O_2 saturation.	Venous CO_2 content.	Venous O_2 saturation.	Difference between arterial and venous CO_2 content.	Difference between arterial and venous CO_2 tension.	O_2 capacity.
	vol. per cent	*per cent*	*vol. per cent*	*per cent*	*vol. per cent*	*mm. Hg*	*vol. per cent*
1	51.8	97.5	57.2	74.3	5.4	8.8	23.7
2	54.7	100.0	56.7	84.9	2.0	2.1	17.2
3	52.9	94.3	55.9	64.4	3.0	6.0	16.3
4	46.5	96.3	51.7	67.1	5.2	8.0	20.6
5	44.8	95.1	48.3	80.8	3.5	6.5	18.7
6	49.7	96.0	54.6	61.6	4.9	6.3	20.6
7	48.1	96.3	52.2	75.0	4.1	7.8	14.4
8	50.6	97.6	58.7	64.0	8.1	13.3	24.7
9	53.3	94.4	60.4	73.5	7.1	14.5	18.9
10	44.6	99.0	50.9	61.8	6.3	8.5	21.2
Average					5.0	8.3	
Maximum					8.1	14.5	
Minimum					2.0	2.1	

the blood. Furthermore, it has already been shown that the air obtained by the Haldane method is not necessarily in equilibrium with the arterial blood, but may be as much as 9.5 mm. of CO_2 tension too low. In this case the values for the venous CO_2 tension obtained by the Henderson method must also be too low, because the two have been shown to differ by only 4 to 7 mm.

Three cases are hardly enough from which to come to very definite conclusions, but it is possible, for general purposes, at least, to use Harrop's figures. Harrop presents the carbon dioxide

content and oxygen unsaturation of both arterial and venous blood from ten normal persons. He gives no absorption curves so that it is impossible to estimate actual arterial and venous tensions from his data. We can, however, deduce with little error the difference between arterial and venous tension by assuming an average slope of 4 volumes per cent of CO_2 for each 10 mm. change in CO_2 tension at tensions that exist in the body. (The variations observed between 30 and 80 mm. are 5.75 to 3.53 volumes per cent per 10 mm., the curves gradually approaching the horizontal as the tension increases.) Assuming such an average slope and employing our formula for correction for oxygen unsaturation we arrive at the results shown in Table VIII. The difference between arterial and venous CO_2 content is from 2.0 to 8.1 volumes per cent, with an average of 5.0 volumes per cent. The difference in tension produced by such a difference in content is 5.0 to 20.0 mm. of Hg uncorrected for oxygen unsaturation, or 2.1 to 14.5 mm. after correction, with an average of 8.4 mm. These values are of essentially the same magnitude as those we obtained.

DISCUSSION.

It has been shown that the alveolar CO_2 tension varies with the height of the absorption curve in such a way that if blood is brought into equilibrium at 37.5°C. with an air-CO_2 mixture of the same CO_2 tension as that of the alveolar air of the subject from whom the blood was obtained, the hydrogen ion concentration of the blood so treated will be found to vary between pH 7.40 and 7.30, with an average of 7.35. At the same time the hydrogen ion concentration of the arterial blood shows a variation twice as great, from pH 7.45 to 7.23. The alveolar and arterial tensions are not identical.

As has been pointed out (28), the alveolar CO_2 tension can be considered from two points of view: (1) As a measure of the arterial CO_2 tension; (2) as an indication of the functional condition of the respiratory mechanism. As a measure of the arterial CO_2 tension it seems to be of less value than it has been commonly considered. But this detracts nothing from its value as an indication of the functional state of the respiratory mechanism. It still remains the effective respiratory air. The real

significance of the fact that the CO_2 tension of this "effective air" varies with the height of the absorption curve may be better appreciated if it is stated in terms of effective ventilation. In this sense it is the portion of the respired air which effects the exchange of gases between the blood and the outside air. From the standpoint of carbon dioxide it is the portion of the expired air that effects the necessary CO_2 elimination. A knowledge of the carbon dioxide output and the alveolar carbon dioxide tension is all that is necessary, therefore, to permit the calculation of the effective ventilation by the formula

$$\frac{\text{Cc. } CO_2 \text{ expired per minute} \times 100}{\text{Per cent } CO_2 \text{ in alveolar air}} = \text{Effective minute volume}^2$$

As the cc. CO_2 expired per minute under resting conditions is comparatively constant, one can say with sufficient accuracy for our purposes that the effective ventilation varies inversely as the alveolar CO_2 per cent. But as the latter varies with the height of the absorption curve, it follows that the effective ventilation must vary inversely as the height of the absorption curve.

This can have but one result: it must tend to maintain the hydrogen ion concentration of the blood at a constant level. Why, then, do we find the variations in arterial pH so great? Because of the slope of the absorption curve small changes in the carbon dioxide content of the blood produce relatively large changes in the carbon dioxide tension. The carbon dioxide content of the blood must be largely dependent upon the carbon dioxide production in the body and must fluctuate with the least change in activity. It would be impossible for the alveolar air to follow these changes immediately and accurately without the destruction of the rhythmicity of respiratory activity. In this case an instantaneous cross-section of the respiratory system such as is presented in these experiments might show considerable temporary disturbances of normal relations. It is inevitable also that these should be most striking in that element of the system which is susceptible to the most rapid changes, the blood; and

[2] The principle on which this formula depends has been employed for estimation of effective ventilation by Campbell, Douglas, Haldane, and Hobson (Campbell, J. M. H., Douglas, C. G., Haldane, J. S., and Hobson, F. G., *J. Physiol.*, 1913, xlvi, 301) and Haggard and Henderson (1).

least evident in the slowest moving portion of the system, the alveolar air.

This would be an entirely satisfactory explanation of temporary differences between alveolar and arterial tension found in single observations; *e.g.*, Case 2. But in Case 3 this difference was found on two different occasions and therefore appeared to be a constant and distinctive characteristic of the subject. The pressure difference necessary to effect the passage of carbon dioxide from the blood in the lungs into the alveolar air is greater than has been supposed, in this case at least. This may mean that the invasion coefficient for CO_2 is not what it has been supposed. It more probably means that the term "alveolar air" has not an exact anatomical significance and that the respiratory air, even from the deeper portions of the lungs, is not of uniform composition and is not all in close communication with the blood in the pulmonary circulation. It may indicate that even in normal persons part of the blood passes through unventilated or incompletely ventilated portions of the lung.

There must be material individual differences in reaction. If the charts of Case 1 and Case 3 (Charts 1 and 3) are compared closely, it is at once evident that every element of the latter lies considerably to the right of the corresponding element of the former. The mechanism seems to be set at a different point. One might say that the respiratory system of Case 3 was relatively insensible to acid. In substantiation of this idea it was found in some previous experiments of a different sort that this subject presented a far greater tolerance to carbon dioxide in rebreathing experiments than did Case 1. The latter exhibited marked distress when the CO_2 of the inspired air rose to about 8 per cent. At this point his respiratory rate became extremely rapid and his tidal air was maximal. At 8.5 per cent of CO_2, Case 3 had increased his tidal air to a maximum, but his respiratory rate was almost unchanged and he experienced only moderate discomfort.

Although it has been demonstrated that the difference in CO_2 tension between arterial and venous blood is very variable and may be larger than was indicated by indirect methods, the corresponding difference in pH is astonishingly small. In Case 3, May 14 (Chart 3, Experiment 2), the difference in carbon dioxide

tension even after correction for oxygen unsaturation was 9.5 mm. of Hg. The corresponding difference in pH was only 0.01. This is, of course, partly due to the slope of the absorption curve but far more to the effect of oxygen and the slope of the pH lines. When arterial blood is changed to venous blood the carbon dioxide increase must bear a definite relation to the oxygen reduction. It appears from the charts that, in persons with a normal hemoglobin, these two factors very nearly equalize one another. That the effect of oxygen on the carbon dioxide absorption curve should tend to diminish the difference in hydrogen ion concentration between arterial and venous blood has already been pointed out by Christiansen, Douglas, and Haldane (9), Hasselbalch (10), Parsons (12), and L. J. Henderson (11). The quantitative relations of the reaction have not been previously established.

By comparison of the charts of Cases 1 and 3 it appears that the ability to maintain the hydrogen ion concentration unchanged in the face of alterations in the carbon dioxide is a variable one. Thus Case 1, May 19 (Chart 1, Curve 2), for a change of 0.8 mm. in CO_2 tension shows no change in the pH; while Case 3, May 14 (Chart 3, Experiment 2), for 9.5 mm. of CO_2 tension shows a change of only 0.01 in pH. This is partly due to the fact that the pH lines, passing through the origin, become more nearly horizontal as the pH diminishes. In order to compensate for large carbon dioxide changes it is of advantage to work at a low pH, or, in terms of the charts, at the right of the chart. This advantage is further increased by the fact that the curves more nearly approach the horizontal as they pass to the right, so that the effect of oxygen is much more marked. This may be a further explanation of the greater tolerance of Case 3 for carbon dioxide in the inspired air.

Haggard and Henderson (14) have taken the position that oxygen is without effect on the carbon dioxide absorption curve of blood and have placed their arterial points accordingly. The close agreement of the values obtained by Michaelis (21) on venous blood, by Hasselbalch (10) on blood equilibrated with alveolar CO_2, and by us on arterial blood is perfectly incomprehensible if Henderson is right. The average difference in carbon dioxide content of arterial and venous blood of normal resting subjects as determined by Harrop (25) and the authors from thirteen persons

is 5.5 volumes per cent. This would give a difference of 0.2 in pH on any normal curve. If the arterial pH is 7.4 to 7.2, the venous pH would then have to be 7.2 to 7.0, far below any values actually found.

Miscellaneous Cases.

Although our interest lay primarily in the study of cardiac decompensation and anemia, two patients with other conditions were subjected to complete experiments. As they belong to neither of our special pathological groups they are reported here. One of these had a moderate polycythemia without splenomegaly, Case 4 (Chart 4). This was not sufficient to alter his absorption curve in a very striking manner, although it shows a slope slightly in excess of the normal. In other respects his chart is indistinguishable from that of a normal person. Again we may note a difference of 10 mm. between the alveolar and arterial carbon dioxide tensions, in spite of the absence of signs of pulmonary and cardiac disease.

Chart 5 is obtained from a patient with advanced chronic nephritis and hypertension. His curve lies at the lowest limit of the normal range and may indicate some reduction of the alkali of the blood. In spite of Cheyne-Stokes respirations there is no evidence of any retention of carbon dioxide, as the arterial pH is 7.37. The arterial-venous difference in carbon dioxide is surprisingly small.

In both cases arterial and venous pH are identical.

SUMMARY AND CONCLUSIONS.

The carbon dioxide absorption curve of human blood from three normal subjects has been determined.

The limits of variation of curves obtained from the blood of normal persons agree with those reported by previous observers. The limits of the carbon dioxide capacity of normal blood at 40 mm. of CO_2 tension and 37.5°C. are 43 and 56 volumes per cent with an average of 49 volumes per cent. Curves of different individuals vary in height and to a lesser extent in pitch. The curve of any given individual is quite characteristic and remains constant over a considerable period of time, as has been previously demonstrated by Christiansen, Douglas, and Haldane.

A direct method for the estimation of the arterial and venous carbon dioxide tension of human blood is described. It has been employed on three normal subjects at rest. The hydrogen ion concentration of arterial and venous blood has been calculated by means of the H_2CO_3:$BHCO_3$ ratio. At the same time the alveolar carbon dioxide tension has been determined by the method of Haldane and Priestley and compared with the arterial carbon dioxide tension found by the direct method.

1. The alveolar carbon dioxide varies, in general, with the height of the absorption curve in such a way that the hydrogen ion concentration of the blood of an individual equilibrated with an air-CO_2 mixture of the same CO_2 tension as that of his alveolar air, will fall between the limits of pH 7.42 and 7.29.

2. Although the relation of alveolar CO_2 tension to height of absorption curve shows this variation in a study of a number of persons, it is constant and characteristic for any one individual.

3. The arterial carbon dioxide tension shows about twice as great a range of variation in relation to the height of the absorption curve. In consequence the arterial pH varies from 7.23 to 7.45. The pH of any one individual is constant and characteristic.

4. The alveolar CO_2 tension is not always the same as the CO_2 tension of the arterial blood. It may be as much as 10 or 11 mm. lower.

5. The carbon dioxide tension of venous blood from an arm vein is higher than the values which have been obtained by the rebreathing methods for mixed venous blood. The difference between arterial and venous tension is variable. It averaged 6 mm. but may be as low as 0.8 mm. or as high as 10 mm. Calculations based on the figures for CO_2 content of arterial and venous blood given by Harrop substantiate our results.

6. Values have been corrected for the effect of oxygen unsaturation on the carbon dioxide-combining capacity of the blood. An empirical equation for such correction is presented.

7. In spite of the large difference between arterial and venous carbon dioxide tension, the pH of the two is almost identical largely because of the effect of oxygen on the carbon dioxide-combining power of the blood. It is pointed out that unless such a mechanism were present to reduce fluctuations in hydrogen ion concentration, the values found by other observers for the pH of venous blood would be impossible.

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EXPLANATION OF CHARTS.

In all charts the ordinates represent the total carbon dioxide content of the blood, expressed in volumes per cent of gas, dry, at 0°, 760 mm. The abscissa represents carbon dioxide tension in mm. of mercury.

That portion of the absorption curve which was experimentally determined is indicated by a solid black line. Extrapolated portions of the curve are indicated by broken lines. In all the pathological cases the limits of variation of the normal absorption curve are shown enclosed by broken lines.

The pH values are calculated by means of the formula of Y. Henderson.

The arterial points are shown as solid circles. The values uncorrected for oxygen are marked A; the corrected values are marked A'. The venous points are indicated by solid squares. The uncorrected points are marked V, the corrected points V'.

The alveolar carbon dioxide tension is indicated in those instances in which it was obtained. The plasma carbon dioxide-combining capacity is also shown in some cases, expressed in terms of the alveolar carbon dioxide tension with which it should agree in normal persons, according to Van Slyke. The case numbers and chart numbers follow the numbering of the protocols.

CHART 1.

Case 1. J. P., normal subject.

Experiment 1. Feb. 27, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	73.7	47.5	15.9
CO_2, *vol. per cent*	64.9	57.7	37.1
	66.5	56.9	37.6

CO_2 content of venous blood, *vol. per cent* 57.1
57.5

Experiment 2. Mar. 5.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	20.3	40.5	65.6
CO_2, *vol. per cent*	39.1	53.7	64.0
	40.0	56.3	

CO_2 content of venous blood, *vol. per cent* 64.3
64.3

Experiment 3. Mar. 12.

Minute volume of respirations, *cc* 7,150
Tidal air 520 cc. Respirations per min 13.7
CO_2 in expired air 4.29 per cent. CO_2 output per min., *cc.* 306
Alveolar CO_2 before determining minute volume, *mm* 41.4
" CO_2 " arterial puncture, *mm* 39.6
" CO_2 after " " " 37.7

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	74.1	47.6	12.2
CO_2, *vol. per cent*	64.3	58.7	34.7
	67.4		

CO_2 content of arterial blood, *vol. per cent* 54.0
54.2

CO_2 " " venous " " " " 62.2
61.7

Difference between arterial and venous CO_2 content, *vol. per cent* 7.9

CO_2 capacity of venous plasma (50.2 mm. Hg), *vol. per cent* 73.0

O_2 content of arterial blood, *vol. per cent* 19.8

O_2 capacity of blood, *vol. per cent* 22.5

O_2 saturation of arterial blood, *per cent* 88

Experiment 4. May 19.

Alveolar CO_2 before arterial puncture, *mm* 38.2
" CO_2 after " " " 37.2
37.0

CO_2 content of arterial blood, *vol. per cent* 54.4
53.7

CO_2 " " venous " " " " 55.7
55.5

Difference between arterial and venous CO_2, *vol. per cent*.	1.5
O_2 capacity of blood, *vol. per cent*	22.1
	22.5
O_2 content of arterial blood, *vol. per cent*	21.5
O_2 " " venous " " " "	18.3
	18.7
O_2 consumption, *vol. per cent*	3.0
O_2 saturation of arterial blood, *per cent*	96
O_2 " " venous " " "	83
Respiratory quotient of blood	0.50

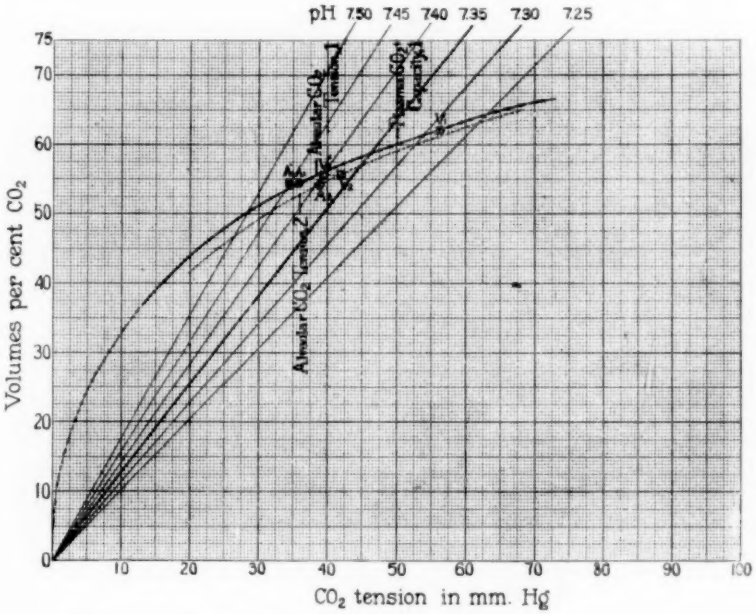

CHART 1. 1. Experiment of Mar. 12.

2. Experiment of May 19. The absorption curve was not determined with this experiment. The absorption curve employed is the average of three determinations made on J. P. on different occasions.

CHART 2.

Case 2. W. S. M. Normal subject.
Experiment 1. Feb. 13, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	13.7	80.6	52.1
CO_2, *vol. per cent*	32.5	66.6	54.5
	35.5	70.0	

CO_2 content of venous blood, *vol. per cent* 63.8
64.3

Experiment 2. May 21. The arterial puncture was rendered difficult by an arterial anomaly. Three punctures were necessary before blood was obtained, causing considerable pain and discomfort. The arm was held rigid during the venous puncture.

Alveolar CO_2 before arterial puncture, *mm* 33.5
" CO_2 after " " " 34.3
36.7

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	94.0	60.2	36.3
CO_2, *vol. per cent*	68.1	56.6	47.2
	68.3		46.1

CO_2 content of arterial blood, *vol. per cent* 50.5
51.3
CO_2 " " venous " " " " 58.9
59.9
Difference between arterial and venous CO_2, *vol. per cent* ... 8.5
O_2 capacity of blood, *vol. per cent* 24.9
O_2 content of arterial blood, *vol. per cent* 21.9
O_2 " " venous " " " " 5.8
O_2 consumption of blood, *vol. per cent* 16.1
O_2 saturation of arterial blood, *per cent* 88
O_2 " " venous " " " 23
Respiratory quotient of blood 0.53

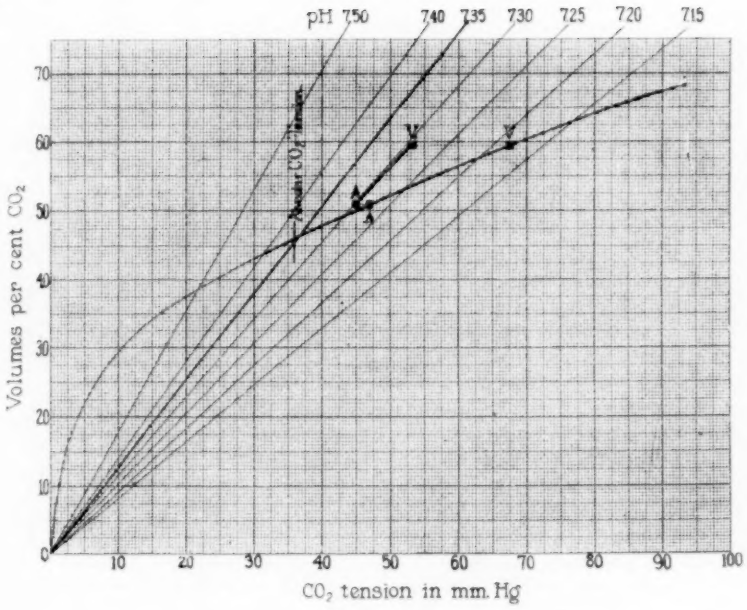
pH 750
740
735
730
725
720
715
Volumes per cent CO_2
CO_2 tension in mm. Hg
0
10
20
30
40
50
60
70
80
90
100

CHART 2.

CHART 3.

Case 3. D. P. B. Normal subject.

Experiment 1. Mar. 26, 1920.

Alveolar CO_2 before arterial puncture, *mm*			38.5
			38.8
" CO_2 after " " "			38.2
CO_2 absorption curve:			
CO_2 tension, *mm. Hg*	85.1	45.0	8.9
CO_2, *vol. per cent*	60.9	45.4	25.1
	61.3		26.0
CO_2 content of arterial blood, *vol. per cent*			45.1
			45.9
CO_2 " " venous " " " "			53.1
			53.6
Difference between arterial and venous CO_2, *vol. per cent*			7.8
CO_2 capacity of venous plasma (43.3 mm. Hg) " " "			62.9

Experiment 2. Mar. 29.

Alveolar CO_2 before venous puncture, *mm*			39.5
			38.7
" CO_2 after " " "			36.0
CO_2 absorption curve:			
CO_2 tension, *mm. Hg*	70.1	41.5	20.1
CO_2, *vol. per cent*	59.9	45.2	36.7
	59.9	47.0	37.9
CO_2 content of venous blood, *vol. per cent*			57.7
			57.3
CO_2 capacity of venous plasma (45.4 mm. Hg), *vol. per cent*			65.9

Experiment 3. May 14.

Alveolar CO_2 after arterial puncture, *mm*			37.4
CO_2 absorption curve:			
CO_2 tension, *mm. Hg*	45.2	23.5	96.0
CO_2, *vol. per cent*	45.3	35.0	61.7
	46.1	35.2	61.9
CO_2 content of arterial blood, *vol. per cent*			47.6
			48.1
CO_2 " " venous " " " "			56.2
			56.2
Difference between arterial and venous CO_2, *vol. per cent*			8.3
O_2 capacity of blood, *vol. per cent*			21.4
O_2 content of arterial blood, *vol. per cent*			19.3
O_2 " " venous " " " "			6.9
O_2 consumption, *vol. per cent*			12.4
O_2 saturation of arterial blood, *per cent*			90
O_2 " " venous " " "			32
Respiratory quotient of blood			0.67

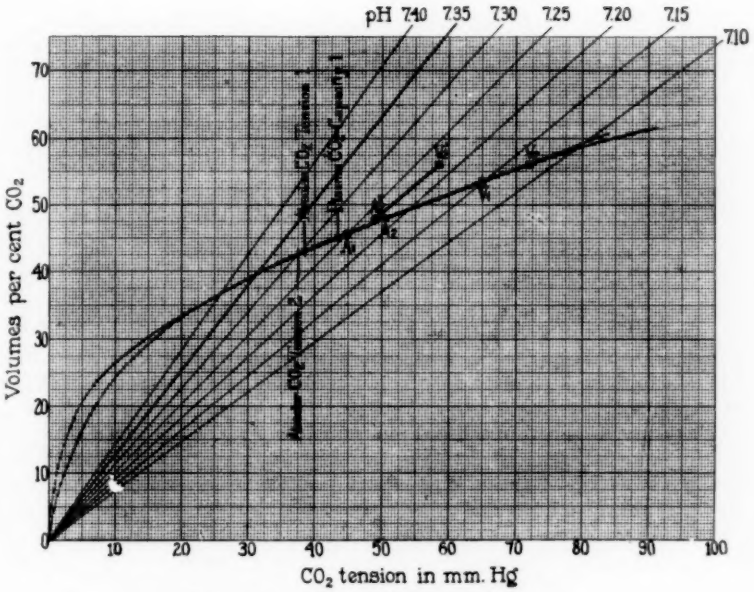

CHART 3. 1. Experiment of Mar. 26.
2. Experiment of May 14.

CHART 4.

Case 4. H. R. Polycythemia. Male, age 36 years. Russian, fireman. History of attacks of dizziness and fainting at intervals for 18 months. For 1 year slight dyspnea on exertion and sharp pains in muscles. Feels lethargic and sleepy in the day time, but restless and wakeful at night. Has to get up two or three times every night to urinate.

Very large man with florid complexion, whose respirations seem somewhat labored. Does not appear to be ill and lies quietly in bed. Tonsils very large and ragged. Lungs clear.

Blood Wassermann negative. Urine negative on two examinations. Fasting blood sugar 130 mg. per 100 cc. 2 hours after 100 gm. glucose by mouth, the blood sugar was still 235 mg. per 100 cc. and he excreted sugar continuously for 24 hours.

Several blood counts gave red blood cell figures varying from 7,800,000 to 8,100,000 in both venous and finger blood. Temperature and pulse normal.

Experiment 1. Apr. 12, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	76.5	67.9	53.6	5.4
CO_2, *vol. per cent*	60.6	56.8	50.3	20.7
		58.6		

O_2 capacity of blood, *vol. per cent* 23.4

Experiment 2. Apr. 28. Has been on a low calory diet with low carbohydrate for 1 week. Respirations quieter than at last observation. Slight cyanosis of finger-tips.

Alveolar CO_2 before arterial puncture, *mm* 34.7
35.9
" CO_2 after " " " 33.6

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	50.2	18.5
CO_2, *vol. per cent*	54.6	31.8
	52.8	32.9

CO_2 content of arterial blood, *vol. per cent* 50.9
49.9
CO_2 " " venous " " " " 60.5
59.9

Difference between arterial and venous CO_2, *vol. per cent* ... 9.8
CO_2 capacity of venous plasma (47.7 mm. Hg), *vol. per cent*. 68.6
O_2 " " blood, *vol. per cent* 23.9
O_2 content of arterial blood, *vol. per cent* 22.6
O_2 " " venous " " " " 8.1
O_2 consumption, *vol. per cent* 14.5
O_2 saturation of arterial blood, *per cent* 95
O_2 " " venous " " " 38
Respiratory quotient of blood 0.68

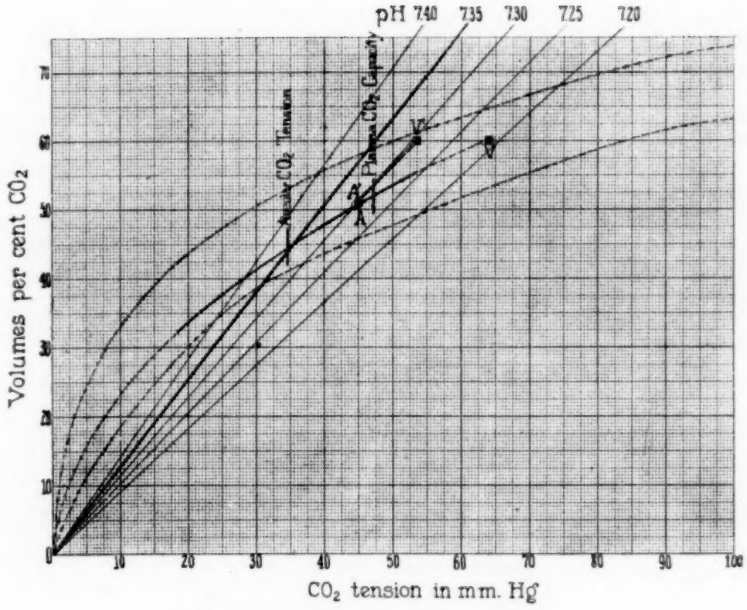

CHART 4. Experiment of Apr. 28.

CHART 5.

Case 5. H. G. Chronic cardionephritic. Male, age 60 years, real estate agent. Looks chronically ill. Quite pale. Cheyne-Stokes breathing with very short periods of apnea. Spastic paralysis of left leg and weakness of muscles of right side of face. Somewhat irrational with loss of emotional control.

Fine râles over left lung at end of inspiration. Heart enlarged to the left, with diastolic murmur over the aortic area and at the apex. Frequent extra systoles. Pulse 64 to 84. Blood pressure: systolic 160, diastolic 90 (has been above 200). Urine shows trace of albumin, no casts. Specific gravity 1.008. Non-protein nitrogen of blood 53 mg. per 100 cc.

Blood count: 4,700,000 red blood cells.

Experiment 1. May 24, 1920. Moderate cyanosis of face and hands. Some dyspnea with Cheyne-Stokes breathing. Arterial blood withdrawn during about equal portions of dyspneic and apneic periods.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	82.1	28.6	45.3
CO_2, *vol. per cent*	54.8	38.9	46.9
	53.5	38.3	48.8

CO_2 content of arterial blood, *vol. per cent*	40.4
	41.4
CO_2 " " venous " " " "	42.7
	44.0
Difference between arterial and venous CO_2, *vol. per cent*	2.4
CO_2 capacity of venous plasma (36.6 mm. Hg), *vol. per cent*	53.3
O_2 " " blood, *vol. per cent*	16.4
O_2 content of arterial blood, *vol. per cent*	13.8
O_2 " " venous " " " "	9.3
O_2 consumption of blood, *vol. per cent*	4.5
O_2 saturation of arterial blood, *per cent*	84
O_2 " " venous " " "	57
Respiratory quotient of blood	0.53

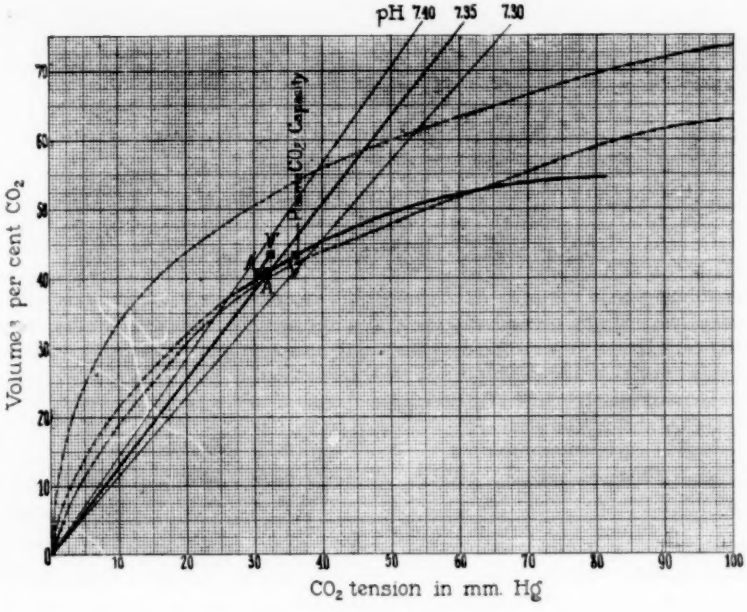

CHART 5.

Miscellaneous Protocols.

Case 21. Jno. K. Diabetes mellitus. Male, age 21 years. Diabetes mellitus of about 7 months duration, moderately severe. Admitted Apr. 5, 1920, with blood sugar of 256 mg. per 100 cc. and marked glycosuria. Emaciated. Skin dry. Cheeks flushed. No dyspnea nor hyperpnea. No cyanosis. Heart and lungs negative to physical examination.

Experiment 1. Apr. 6.

Alveolar CO_2, *mm*	30.0
	29.7
	30.8

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	82.9	68.1	32.7	14.1
CO_2, *vol. per cent*	58.2	53.1	37.6	27.5

CO_2 capacity of venous plasma (34.9 mm. Hg), *vol. per cent*.. 50.7

O_2 " " blood, *vol. per cent*. 19.2

Case 22. I. M. Diabetes mellitus. Male, age 52 years, married, suit presser. Diabetes of about 7 months duration, rather mild. Well nourished and developed. No dyspnea or hyperpnea. No cyanosis. Blood sugar 202 mg. per 100 cc. Heart negative. Blood pressure: systolic 140,

diastolic 88. Lungs show few, scattered, moist râles. Discharged from hospital sugar-free on diet containing 1,650 calories.

Experiment 1. Apr. 14, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	71.1	18.1	58.4
CO_2, *vol. per cent*	56.1	29.4	51.1
	59.0	32.3	

CO_2 capacity of venous plasma (40.9 mm. Hg), *vol. per cent.* 59.5

O_2 " " blood, *vol. per cent* 24.4

Case 23. I. S. Diabetes mellitus. Male, age 42 years, married, cutter. Diabetes of moderate severity for 2½ years. Emaciated, drowsy. No visible signs of dyspnea or hyperpnea. No cyanosis. Lungs and heart negative. Slight pitting edema of the ankles. Blood sugar 274 mg. per 100 cc. Urine shows considerable sugar and acetone. Temperature and pulse normal.

Experiment 1. Mar. 31, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	76.0	49.3	28.3
CO_2, *vol. per cent*	62.0	49.3	39.5
		48.0	38.5

CO_2 capacity of venous plasma (43.7 mm. Hg), *vol. per cent.* 63.5

O_2 " " " blood, *vol. per cent* 17.9

18.2

Case 24. D. A. Diabetes mellitus with vulvar abscess. Old woman with senile dementia and diabetes of long standing, complicated by an extensive cellulitis of the vulva. Breathing quietly. Slightly cyanotic. Blood sugar 333 mg. per 100 cc.

Experiment 1.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	78.4	42.3	17.8
CO_2, *vol. per cent*	61.6	42.4	27.4
	61.5	44.0	28.9

CO_2 content of venous blood, *vol. per cent* 56.8

57.5

CO_2 capacity of venous plasma (46.8 mm. Hg), *vol. per cent.* 68.0

O_2 " " blood, *vol. per cent* 23.8

Case 25. E. C. Emphysema with slight cardiac decompensation.

Experiment 1.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	85.2	25.5
CO_2, *vol. per cent*	70.2	44.5
	68.8	44.6

O_2 capacity of blood, *vol. per cent* 22.3

22.4

II. THE CARBON DIOXIDE ABSORPTION CURVE AND CARBON DIOXIDE TENSION OF THE BLOOD IN CARDIAC DYSPNEA.

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INTRODUCTION.

In cardiac dyspnea the carbon dioxide tension of the alveolar air as determined by the Haldane method is lower than normal in comparison with the plasma bicarbonate as measured by means of the Van Slyke pipette (1, 2). The ratio between these two in health and in most pathological conditions has been shown by Van Slyke, Stillman, and Cullen (3), Walker and Frothingham (4), and Peters (1, 5) to be very close, varying from the mean by only 10 per cent. The existence of such a close relation might well be expected in the light of current theories with regard to the physicochemical control of the respiratory mechanism. That this relation should be disturbed in cardiac dyspnea is hardly extraordinary.

In the average normal person at rest carbon dioxide content, carbon dioxide tension, and hydrogen ion concentration of arterial and of venous blood all vary within comparatively narrow limits both individually and in relation to one another. Furthermore, the physicochemical regulatory mechanism is so delicately adjusted that changes in any of these factors are reflected in an immediate response on the part of the respiratory mechanism, which produces a compensatory alteration of the alveolar CO_2. As long as the sensibility of the respiratory center and the mechanical facilities for the exchange of gases between the blood and the air in the lungs remain unaffected, changes in any of these factors are instantly compensated by changes in one or all of the others. If either the

sensibility of the respiratory center or the mechanical facilities for the elimination of CO_2 in the lungs are impaired, one or all of the normal interrelations must be disturbed.

In cardiac decompensation, with dyspnea, there is evidence that the efficiency of the lungs as a means for the oxygenation of the blood and the elimination of CO_2 is greatly reduced (2, 6). There is also a possibility that the circulation is retarded.

In an attempt to throw some light on the exact cause of the discrepancy between alveolar and plasma values we first devoted our attention to a study of those factors of the respiratory system that may be termed mechanical: the function of the lungs and the upper respiratory tract. The results of this study have been published. It was found that air obtained by the Haldane method from patients with cardiac dyspnea was comparable in a functional sense to that obtained from trained normal subjects (2). That is, the air so obtained was the air employed for the exchange of gases between the blood and the outside air and was the only air available for this purpose. Realizing the objections that might be raised to the use of the term "alveolar" air in this sense, we suggested the terms "effective" or "exchange" air. By this means we evaded the criticisms which had been advanced by Siebeck (6). The latter, in the course of some studies of the lung volume in cardiac decompensation, came to the conclusion that the lungs in this condition failed to function efficiently as a tonometer. He decided that the mixing of gases in the lungs was imperfect and that methods for obtaining alveolar air were inapplicable. However, he considered the alveolar air only as a means of ascertaining the carbon dioxide tension of the arterial blood. Although our findings were not entirely in accord with Siebeck's, no attempt to controvert his statement was made. The question of the relation of alveolar CO_2 to arterial tension was entirely ignored, although its importance was fully recognized. Further studies convinced us that the effective lung volume was diminished (7) and the effective ventilation increased (8) in cardiac decompensation. Pearce (9) had proposed as an explanation of the disturbance of the alveolar: plasma ratio a retarded circulation. In view of the changes in the lung volume and the findings of Siebeck, however, this could not be definitely proved by the ordinary respiratory methods. Pearce assumed what Siebeck denied,

the applicability of alveolar methods as a means of determining arterial CO_2 tension. Even in normal subjects such an assumption seemed to be open to some criticism. The only direct evidence of a definite relation between alveolar and arterial CO_2 tension was given by Krogh and Krogh (10) in some tonometric experiments on animals. There was no evidence of any kind that a similar relation existed in diseases involving disturbances of the respiratory mechanism. Obviously it was necessary to find a means to measure the arterial CO_2 tension and to compare this with the alveolar CO_2 tension. For this reason the method described in the first paper was employed in the study of patients with cardiac decompensation. The CO_2 absorption curves of seven patients were obtained one or more times. Complete experiments were made on four cases out of seven. The results of these experiments are collected in Table III, arranged as were those of the normal subjects in Table VI of Paper I. Detailed protocols of all seven cases are presented at the end of this paper.

Level of the Absorption Curve in Cardiac Dyspnea.

The absorption curves of normal individuals fall within certain definite limits and are constant and characteristic for any given individual. This is far from the case in pathological subjects. The variations in both group and individual are considerable. The limits of normal variations in height are about 43 to 56 volumes per cent. The limits of variation of twenty-two patients with various pathological conditions were 32.4 to 70.2 volumes per cent, about three times as great. It is probable that a study of more patients with a greater variety of pathological conditions would show a still greater range of variation. In relatively few cases has more than one observation been made; but in these few the changes in height at different times are also quite striking. The maximum change observed was in Case 11, whose curve rose from 36.2 to 51.1 in the course of 4 days. Such fluctuations are not restricted to conditions of recognized acidosis, such as is found in diabetes mellitus or in nephritis. They occur also in cardiac valvular disease and in severe anemia.

The height of the absorption curves of seven decompensated cardiac patients, on whom eleven observations were made, varied from 32.4 to 52.0 volumes per cent, with an average value of 44.7

TABLE I.

Absorption Curve at 40 Mm. CO_2 Tension in Seven Patients with Cardiac Decompensation and Dyspnea.

Case.	Diagnosis.	CO_2 content.
		vol. per cent
6. G. B.	Paroxysmal tachycardia. Some dyspnea; considerable cyanosis; fluid in right pleural cavity and in abdomen; edema of both ankles; temperature 103°.	45.4
7. J. D. B.	Aortic regurgitation. Mar. 19. Able to be up and about ward, but showed moderate dyspnea and orthopnea, with faint cyanosis of lips and finger-tips even while at rest.	45.0
	Apr. 30. Condition practically unchanged.	47.5
8. E. H.	Mitral stenosis; auricular fibrillation. Marked dyspnea and orthopnea; considerable cyanosis; general edema; hydrothorax and ascites.	47.5
11. J. K.	Mitral stenosis; auricular fibrillation.	
	Apr. 16. Extreme cyanosis; moderate hyperpnea and edema.	36.2
	Apr. 20. Cyanosis and hyperpnea improved.	51.1
10. J. M.	Mitral stenosis; auricular fibrillation.	
	Apr. 9. Extreme cyanosis; moderate dyspnea and edema.	38.7
	Apr. 23. Cyanosis continues; dyspnea and edema gone; still some hyperpnea.	48.1
9. P. O. S.	Aortic regurgitation.	
	Mar. 9. Marked dyspnea with irregular breathing. Moderate cyanosis; considerable edema with hydrothorax.	52.0
	Mar. 23. Symptoms steadily increasing.	48.1
12. C. C.	Mitral stenosis. Marked cyanosis; moderate hyperpnea.	32.4
Maximum.		52.0
Minimum.		32.4
Average.		44.7

volumes per cent (see Table I and Fig. A). Of the seven patients three showed a definite reduction of the alkali reserve of the blood during the stage of most severe decompensation. Later observations on two of these, at the time when compensation was becoming reestablished, gave normal values. The other four cases showed no departure from the normal as regards the height of their curves.

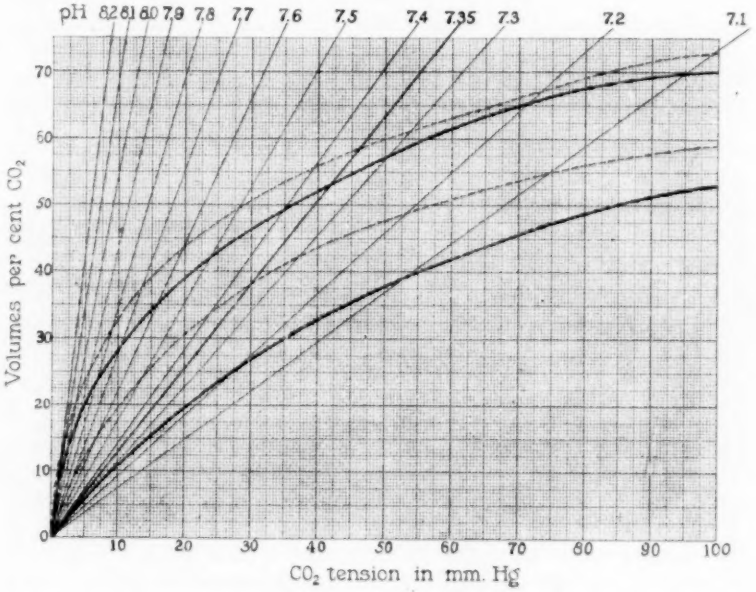

FIG. A. A comparison of limits of absorption curves of normal and cardiac subjects.

Limits of cardiac curves.

Limits of normal curves.

A low absorption curve is not a characteristic of the condition of cardiac decompensation. What factors determine the presence of such a curve in certain cases is not clear. As we shall show later, there is a relative retention of carbon dioxide in the arterial and venous blood of the cases with acidosis. This, in itself, should tend to produce an increase rather than a reduction of the alkali reserve, according to Henderson and Haggard (11).

It has been demonstrated by several observers, using various methods, that reduction of the partial pressure of oxygen in the blood produces a reduction of carbon dioxide tension and carbon dioxide content of the blood (12–15). However, in these studies the reduction of oxygen tension was produced in such a way that CO_2 was not allowed to accumulate in the blood. Whether a similar reduction in the level of the absorption curve will be produced by low oxygen in the presence of CO_2 accumulation has not been determined. That there is a certain amount of oxygen

TABLE II.

Comparison of CO_2 Capacity of Whole Blood and Oxygen Unsaturation of Blood.

Subject and diagnosis.	Date.	CO_2 capacity of blood.	Oxygen capacity of blood.	Oxygen content.		Oxygen saturation.	
				Arterial blood.	Venous blood.	Arterial blood.	Venous blood.
	1920	vol. per cent	vol. per cent	vol. per cent	vol. per cent	per cent	per cent
1. J. P. Normal.....	Mar. 12	55.9	22.5	19.8		88.0	
2. D. P. B. "	May 14	43.6	21.4	19.3	6.9	90.2	32.2
3. W. S. M. "	" 21	48.2	24.9	21.9	5.8	88.0	23.3
4. M. C. Asthma and emphysema.....	" 12	49.0	21.3	17.2	11.0	80.8	51.7
5. H. R. Polycythemia.	Apr. 28	47.9	23.9	22.6	9.0	94.6	37.7
6. J. K. Cardiac.....	" 20	51.1	26.5	25.0	22.5	94.3	84.9
7. J. M. "	" 23	48.1	20.8	22.0	11.0	100.0	52.9
8. J. D. B. "	" 30	47.5	13.9	14.3	3.7	100.0	26.6
9. G. B. "	Mar. 24	45.4	21.7		5.0		23.0
10. J. M. "	Apr. 9	38.7	20.2	16.7	8.5	82.7	42.1
11. J. K. "	Mar. 16	36.2	26.5	24.2	9.9	91.3	37.4
12. C. C. "	Apr. 9	32.4	18.3	18.0	3.2	98.4	17.5

unsaturation in the arterial blood and a very marked degree of oxygen unsaturation in the venous blood of some patients with cardiac decompensation has been shown by Harrop (16). As the most notable feature of the three patients with low absorption curves was a degree of cyanosis out of all proportion to the degree of dyspnea and hyperpnea, it seemed possible that the height of the absorption curve might bear some relation to the degree of oxygen unsaturation of the arterial and the venous blood.

That no such relation can be clearly established in our cases is evident from Table II, in which are shown the height of the carbon

dioxide absorption curve, and the oxygen content and saturation of both arterial and venous blood. At least, if the low curve is due to oxygen unsaturation, this is not the only active factor.

Although the restoration of compensation was associated with a return of the absorption curve to its normal level in Cases 10 and 11, the height of the absorption curve is no indication of the severity of the cardiac decompensation. The three cases with low curves were discharged from the hospital improved, while Cases 6, 8, and 9 with normal curves, failed to improve and died shortly after the observations reported.

There was nothing in the clinical picture to suggest a nephritic acidosis in any of these cases as the cause of the low absorption curves. Rather complete renal functional studies were made in Case 10. His blood non-protein nitrogen was only 43 mg. per 100 cc. and his phenolsulfonephthalein excretion 60 per cent in 2 hours.

We are then left to the conclusion that cardiac decompensation is sometimes associated with a real reduction of the alkali reserve of the blood, which disappears when compensation is reestablished. Why this should occur in some instances and not in others is not explained.

Arterial and Venous Carbon Dioxide Tension and Hydrogen Ion Concentration and Alveolar Carbon Dioxide Tension in Cardiac Dyspnea.

Complete experiments were made on four out of the seven cases with cardiac decompensation. The results of these experiments appear in Table III (arranged as were those of the normal subjects in Table VI, Paper I), and in Charts 7, 10, 11, and 12 inclusive. If the general averages in Table III are compared with those in Table VI, Paper I, the following points of distinction appear:

1. Although there is little difference in the range of variation of arterial CO_2 tension in the two tables, the CO_2 tension during cardiac dyspnea is high in relation to the level of the absorption curve because the absorption curves are low in three of the four subjects.

2. The consequence is that the arterial pH values in cardiac dyspnea are on the average lower than normal, indicating a carbon dioxide retention in the arterial blood.

TABLE III.

Results of the Study of Seven Cardiac Patients with Dyspnœa.

Subject.	Date.	Alveolar CO_2 tension.	Alveolar reaction.	Arterial CO_2 content.	Arterial CO_2 tension.		Arterial reaction.		Venous CO_2 content.	Venous CO_2 tension.		Venous reaction.		Difference between arterial and alveolar CO_2 tension.	Difference between venous and arterial CO_2 content.	Difference between venous and arterial CO_2 tension.	Difference between venous and arterial reaction.
					Uncorrected.	Corrected.	Uncorrected.	Corrected.		Uncorrected.	Corrected.	Uncorrected.	Corrected.				
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
	1920	*mm. Hg*	*pH*	*vol. per cent*	*mm. Hg*	*mm. Hg*	*pH*	*pH*	*vol. per cent*	*mm. Hg*	*mm. Hg*	*pH*	*pH*	*mm. Hg*	*vol. per cent*	*mm. Hg*	*pH*
6. G. B.	Mar. 24								54.8	61.0	47.2	7.19	7.26				
7. J. D. B.	" 19	25.2	7.42						49.8	50.5		7.23					
	Apr. 30	27.6	7.42	49.1	42.5	42.5	7.30	7.30	52.7	53.0	46.5	7.25	7.30	14.9	3.6	4.0	0.00
8. E. H.	Mar. 10								51.1	46.5		7.29					
9. P. O. S.	" 9								62.1	61.0		7.25					
	" 23								56.9	65.5		7.18					
10. J. M.	Apr. 9	26.0	7.34	39.6	40.5	38.0	7.23	7.25	49.8	61.0	52.3	7.15	7.22	13.0	10.2	14.3	0.03
	" 23	33.0	7.37	54.0	52.2	52.2	7.26	7.26	63.4	75.0	65.2	7.16	7.23	19.2	9.4	13.0	0.03
11. J. K.	" 16	22.0	7.32	35.9	39.7	38.0	7.20	7.20	45.5	62.3	47.5	7.10	7.21	16.0	9.6	9.5	−0.01
	" 20*	(32.5)	(7.41)	(52.4)	(42.25)	(41.25)	(7.34)	(7.35)						(8.75)			
12. C. C.		27.4	7.22	33.5	42.5	42.5	7.13	7.13	42.9	62.5	50.0	7.06	7.17	15.1	9.4	7.5	−0.04
Maximum*.....		33.0	7.42	54.0	52.2	52.2	7.30	7.30	63.4	75.0	65.2	7.29	7.30	19.2	10.2	14.3	0.03
Minimum*.....		22.0	7.22	33.5	39.7	38.0	7.13	7.13	42.9	46.5	46.5	7.06	7.17	13.0	3.6	4.0	−0.04

* The values obtained from the experiment of Apr. 20 on J. K. have been omitted in the summary of maximum and minimum

3. The alveolar CO_2 tension is, as has been previously pointed out, lower than normal. It is variable in relation to the absorption curve and may be, as in Case 12 (Chart 12), comparatively high, giving an alveolar pH far below the normal limits.

4. The difference between alveolar and arterial CO_2 tension is consistently increased. In five experiments on dyspneic cardiac patients this difference never fell below 13 mm. and in one case it reached the astonishing figure of 19 mm.

5. The venous CO_2 tension falls within normal limits, but, like the arterial, is high in relation to the level of the absorption curve.

6. There is no consistent increase in the difference between arterial and venous CO_2 content or tension, although in one or two instances both were slightly above the normal limits.

When each of these factors is studied in relation to the individual subjects of these experiments, it is apparent that only one is definitely and consistently present in every instance of cardiac dyspnea: this is the increase in the difference between alveolar and arterial CO_2 tension.

Although the average arterial CO_2 tension is relatively high and the arterial pH, in consequence, lower than normal, in Cases 7 (Chart 7) and 10 (Chart 10) the pH of the arterial blood is quite within normal limits. Can one say, then, that a carbon dioxide retention is characteristic of cardiac dyspnea, or does it occur only in certain cases, possibly those who show a considerable reduction in the height of the absorption curve, as do Cases 11 and 12? Unfortunately the range of variation in the normal arterial pH makes this question hard to answer. Absolute values are of little assistance in judging relative factors. Our results suggest that different normal subjects maintain the pH of their arterial blood at different levels. Furthermore the resting arterial pH level seems to be characteristic and constant for a given individual, as is the level of the absorption curve itself. In this case an arterial pH which is normal for one individual might mean a considerable retention of carbon dioxide in the case of another.

The statement was made above that there was nothing characteristic about the height of the absorption curve of the blood of patients with cardiac dyspnea, but that in certain cases low curves were found, indicating a diminution of the available alkali of the blood. We were then considering the absolute level of the

absorption curve. Again attention must be called to the fact that we are dealing in relative values only and that absolute levels, although satisfactory for purposes of clinical study, give little information with regard to functional changes in physiology or pathology. In order to ascertain whether a given pathological condition has changed the level of either the absorption curve or the arterial pH of an individual, it is not enough to know the limits of variation of normal individuals as a group. It is necessary to know the normal resting absorption curve and arterial pH of the given subject under investigation. In clinical investigations this is not always possible. Patients with severe cardiac dyspnea do not invariably recover. Of our seven cases three died without any intervals of improvement (Cases 6, 8, and 9); one (Case 7) had a chronic cardiac decompensation which resisted treatment, increasing gradually but steadily. Cases 10 and 11 improved. The former never completely recovered compensation; the latter made a very rapid recovery and was discharged without dyspnea or hyperpnea. Two determinations of the absorption curve were made on the blood of four of the seven patients: Cases 7, 9, 10, and 11.

In Case 9 the second observation, made 2 weeks after the first, revealed a drop of 4 volumes per cent in the height of the absorption curve. During this time the condition of the patient had continuously grown worse. The two observations on Case 7, at an interval of almost 6 weeks, were only about 2 volumes per cent apart. The second was the higher. There was little change in Case 7's condition on these two occasions. Possibly his dyspnea was a little more severe in the earlier experiment; his general condition seemed a trifle less favorable at the time of the second observation. The changes in absorption curve and in condition are, in any event, of no significance.

The rise in level of the absorption curves that occurred during the clinical improvement of Cases 10 and 11 is very striking. Although it is impossible, then, to say that cardiac dyspnea is always attended by a lowering of the absorption curve, there is a suggestion that such an association is not uncommon.

For the arterial pH our data are much more meager. This was determined in only four cases and in only two of these were two determinations obtained. In one of these, Case 10, the pH

remained unchanged in spite of a rise in the level of the absorption curve; in the other, Case 11, both pH and absorption curve rose. The latter regained compensation more completely than did the former in the interval between observations. The second experiment on Case 11 gives normal results in all essentials. Even the difference between alveolar and arterial CO_2 tension has returned to normal limits. It is quite conceivable that the picture presented by Case 10 on April 23 represents a preliminary step on the way to compensation and that, with complete recovery, which never occurred, the arterial pH would also have risen. There is no doubt that Case 12 had an uncompensated acidosis because his arterial pH lay below the normal limits. A definite carbon dioxide retention or uncompensated acidosis[1] occurred in two subjects, then. In the others a similar condition cannot be excluded as the result of our experiments although the arterial pH lay within normal limits.

There is nothing questionable about the increased difference between arterial and alveolar CO_2 tension. It occurred consistently in the presence of dyspnea and returned to normal in the one instance in which compensation was definitely reestablished. It is inconceivable that such a difference is due merely to errors in analytical methods or in calculation. It has been shown that the assumption that the alveolar air is in carbon dioxide equilibrium with the arterial blood is not always in keeping with the facts. That such differences as occurred in normal resting subjects might be only temporary was pointed out. But in cardiac dyspnea the differences are far greater and are invariably present.

Such a difference in itself might be produced by a diminution of the rate of the circulation. The venous blood might return to the lungs with an accumulation of carbon dioxide too great to permit complete removal in the lungs. The CO_2 remaining would pass on into the arteries and produce an increase in ventilation by its action on the respiratory center. In this case the difference between arterial and venous CO_2 tension and arterial and venous CO_2 content should be increased. Such an increase

[1] By a carbon dioxide retention or uncompensated acidosis we mean an accumulation of carbon dioxide sufficient to produce a real change in the pH of the blood.

is sometimes found, but it is not consistent and bears no relation to the arterial-alveolar discrepancy. The differences between arterial and venous CO_2 tension and arterial and venous CO_2 content in Case 7, for instance, are only 4.0 mm. and 3.6 volumes per cent respectively, while the arterial-alveolar difference is 14.9 mm. In addition to our own experiments we have calculated the differences between arterial and venous CO_2 content and CO_2 tension in ten of Harrop's (16) cardiac cases who showed definite evidences of decompensation. The calculations were made by the same formula employed in the normal cases (see Paper I). The results appear in Table IV and can be compared with Harrop's normal cases in Table VIII, Paper I. In his cases, as in ours, there is little evidence of a diminished circulation rate. A retarded circulation would seem to be, therefore, at the most only an occasional minor cause of the difference between arterial and alveolar CO_2 tension.

The predominant cause must lie in the lungs themselves. There must be an interference with the escape of CO_2 from the blood in the pulmonary circulation. This seems the more probable because of the known changes in the effective lung volume (7, 17). There may be portions of the lungs in which the circulation is more or less intact, but which contain no air. Or there may be portions of the lungs which are air-containing, but immobile and not adequately ventilated by the respirations. The latter is the view of Siebeck (6). Present methods of measuring lung volume have failed to settle the question because they are capable of measuring only the portions of the lungs that contain air available for respiratory purposes. Whether or not there is a true carbon dioxide retention in all cases of cardiac dyspnea, there is always an interference with the elimination of CO_2 from the blood and, therefore, a compensated or potential acidosis.

If the alveolar air is considered from the standpoint of respiratory mechanics, as a measure of the effective ventilation, a greater effective ventilation is necessary in order to maintain a given arterial carbon dioxide tension in the case of the patient with cardiac dyspnea than is necessary in the case of the normal person. To what extent does this account for the increase in the hydrogen ion concentration of the arterial blood? Does the alveolar carbon dioxide tension always indicate a ventilation in

excess of the normal in relation to the height of the absorption curve? If this were true the alveolar pH should always be considerably above 7.30. This is not true in Case 10, April 9, Case 11, April 16, and Case 12. In these instances the alveolar carbon dioxide is at or below the normal limit in relation to the height of

TABLE IV.

Difference between Arterial and Venous CO_2 Tension.

(Calculated from observations on patients with cardiac decompensation, presented by Harrop.)

Case No.	Arterial CO_2 content.	Arterial oxygen content.	Venous CO_2 content.	Venous oxygen content.	Difference between arterial and venous CO_2 content.	Difference between arterial and venous CO_2 tension.	Oxygen capacity.
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*mm. Hg*	*vol. per cent*
1	46.4	17.2	54.0	6.9	7.6	15.4	20.3
	46.3	19.6	48.7	16.3	2.4	4.9	21.8
	44.3	22.3	56.1	15.5	11.8	27.2	23.1
2	45.4	17.1	48.3	9.3	2.9	4.6	19.0
	48.2	17.9	52.7	9.5	4.5	8.4	18.7
	49.6	17.8	53.1	12.6	3.5	6.9	18.5
4	41.4	13.9	44.2	10.8	2.8	5.9	16.8
	43.5	15.2	46.5	9.6	3.0	5.6	16.6
5	38.9	17.7	41.8	6.3	2.9	3.3	19.9
	42.7	18.2	47.1	10.4	4.4	8.3	19.7
	43.8	17.9	47.6	13.1	3.8	7.8	19.6
7	38.7	16.5	43.2	7.3	4.5	7.5	18.9
	42.9	20.5	46.5	16.1	3.6	7.5	22.9
	43.6	21.1	49.0	15.4	5.4	5.5	22.5
8	31.4	19.3	37.5	11.1	6.1	12.4	20.7
	42.8	21.3	45.7	11.6	2.9	3.9	22.5
	41.5	22.5	46.5	17.6	5.0	10.8	23.3
Maximum..	49.6		56.1		11.8	27.2	
Minimum..	31.4		37.5		2.4	3.3	
Average....	43.0		47.6		4.5	8.6	

the corresponding absorption curves. Although these three patients showed some dyspnea the ventilation was insufficient to compensate for the low absorption curves they presented. Nor was this due to the fact that they had passed the limits of compensation and were unable to increase their ventilation

further. In none of these cases was dyspnea a prominent feature. The actual minute volume of respiration of Case 10 was only 8,650 cc. with a respiratory rate of 18 per minute. This suggests that the uncompensated acidosis found in these cases was not due entirely to an inability of the injured respiratory mechanism to effect the proper carbon dioxide elimination, but to the fact that the respiratory center was not so sensitive as normal to its natural stimulus, the hydrogen ion concentration of the blood. As the comparative lack of dyspnea shown by these patients is not at all typical of cardiac decompensation, this insensibility of the respiratory center to the natural acid stimulus is not necessarily a characteristic of cardiac dyspnea *per se*.

In the normal subjects the pH of the arterial and venous blood was found to be almost identical in spite of a considerable difference in CO_2 content. This was possible because of the effect of oxygen on the absorption curve. The same holds true of the cardiac cases, in some of whom even larger differences of CO_2 content occur. The difference in pH of arterial and venous blood never exceeds 0.04 and is, therefore, within the limits of error of the method.

One may pause for a moment to consider what bearing the presence of a carbon dioxide retention in association with a low absorption curve may have on the work of Henderson and Haggard (11). They claim that any damming back of carbon dioxide causes the blood to abstract alkali from the tissues and produces a rise in the level of the absorption curve. The facts here established for cardiac dyspnea are quite at variance with such a theory unless one argue from a teleological standpoint that the damming back of carbon dioxide is produced in an attempt to withdraw alkali from the tissues and thus restore the alkali of the blood to its normal level. Aside from the fact that this ascribes to the blood a purposeful effort at compensation, this is not the usual reaction to a reduction in the level of the absorption curve as is evidenced by the curves of nephritis patients (see Case 5, Paper I, and Case 16, Paper III).

It is interesting to compare the relations that obtain in cardiacs with those found by Means, Bock, and Woodwell (18) in pneumonia and with those found by us in one case of severe asthma and emphysema with extreme cyanosis (Case 13, Chart 13).

These investigators found a carbon dioxide acidosis in three pneumonia patients, without any reduction in the level of the absorption curve. They, however, accept Henderson's view that oxygen does not affect the absorption curve of blood. Fortunately they have published figures which allow us to make corrections by our formula. As we have calculated their results the arterial pH values in their three cases are: for E. D., 7.23 uncorrected, 7.28 corrected; for G. A., 7.30 uncorrected, 7.35 corrected; for N. D., 7.27 uncorrected, 7.30 corrected. Because of the degree of oxygen unsaturation in the arterial blood of pneumonia patients these corrections are much larger than the corresponding corrections in cardiac dyspnea, where the oxygen unsaturation of the arterial blood is usually comparatively slight. If oxygen does affect the CO_2-combining capacity of blood there is little evidence of a carbon dioxide acidosis in these figures alone.

Case 13 had an asthmatic condition of long standing with an extreme emphysema and the most extraordinary cyanosis. His face and extremities were at all times a deep purple and during his acute attacks were almost black. The experiment was performed during one of these attacks. His absorption curve lies at a normal level. His arterial tension is quite high in proportion and about 18 mm. higher than the alveolar tension, which lies at a pH of 7.33. The corrected arterial pH is 7.22. The most striking thing about his chart is the enormous venous carbon dioxide tension. Although the difference between the arterial and venous carbon dioxide content is only 5.7 volumes per cent, the portion of the absorption curve on which the arterial and venous points lie is so flat that this represents a change of CO_2 tension of about 40 mm. uncorrected, as far as we can tell by extrapolating the venous point. The compensating effect of oxygen is, however, also increased by the flatness of the curve sufficiently to reduce the venous tension about 35 mm. (In calculating the effect of oxygen on the venous point in this case we were unable to use our formula because the CO_2 tension was above 70 mm. A value for K of 0.414 was used instead of the usual 0.34. This is the average value of K between 70 and 90 mm. From Table I it appears that there is a considerable variation in the value of K above 70 mm. and there may be a large error in this correction.) In spite of this there is still a very

considerable difference in pH between the arterial and venous blood. This difference is, however, much less than would occur if the arterial tension were lower on a curve of the same level, because of the difference in the slope of the pH lines. In a study of these factors may lie an explanation of the relatively great tolerance for carbon dioxide found by Scott (19) in emphysema patients. No general conclusions can be drawn from this one patient as to the respiratory mechanism in emphysema or asthma. The picture is similar to that of cardiac dyspnea in the fact that there is a marked difference between the alveolar and the arterial CO_2 tension, suggesting again a functional impairment of the ventilating power of the lungs. Furthermore, there is a carbon dioxide retention, as evidenced by the low arterial pH.

Plasma Bicarbonate as a Measure of the Alkali Reserve.

As a result of a large series of observations of the carbon dioxide-combining capacity of the venous plasma of decompensated cardiac patients made in 1916 and subsequently, we were led to conclude that the alkali reserve of the blood of these patients, although quite variable, lay for the most part within normal limits. The same thing is true of the present cases, if we consider the carbon dioxide capacity of the plasma. Six determinations in this series vary from 55.0 to 75.7 volumes per cent. Among these is a determination made on Case 11 from the same specimen of blood that showed such a marked reduction in the height of the absorption curve.

The contradictory results obtained from whole blood and from venous plasma may be explained by variations in the carbon dioxide content of the venous blood. As the tension of carbon dioxide in blood is increased the bicarbonate of the blood also increases. This is evidenced by the fact that the slope of the absorption curve is greater than that of the curve which represents the solubility of carbon dioxide in the blood. The increase in the bicarbonate of blood in response to increases of CO_2 tension is associated with a transfer of base from cells to plasma. Joffe and Poulton (20) have demonstrated that the change in bicarbonate content of whole blood is reflected in the plasma. This had already been clearly demonstrated by Van Slyke and Cullen (21). Van Slyke and Cullen, Straub and Meier (22), and

others have shown that the CO_2 absorption curve of plasma is much flatter than that of whole blood, approaching more nearly the curve of a simple bicarbonate solution.

In this case the carbon dioxide-combining capacity of the venous plasma must be dependent, to some extent at least, upon the carbon dioxide tension or content of the venous blood at the time that it is withdrawn. Table V shows this relation clearly. In this table are represented the carbon dioxide content of venous blood, drawn directly, without stasis, beneath albolene; the

TABLE V.

Relation between CO_2 Content of Venous Blood and CO_2 Capacity of Venous Plasma.

Subject.	CO_2 capacity of whole blood.	CO_2 content of venous blood.	CO_2 capacity of venous plasma.
	vol. per cent	*vol. per cent*	*vol. per cent*
A. B.	60.8	63.9	69.1
J. M.	48.1	63.3	75.7
M. C.	49.0	63.2	74.5
P. O. S.	52.0	62.1	74.2
J. P.	55.9	62.0	73.0
H. R.	47.7	60.2	68.6
N. B.	52.7	60.1	66.0
D. P. B.	47.0	57.5	66.0
D. A.	42.1	57.2	68.0
D. P. B.	43.3	53.3	62.9
E. H.	47.5	51.1	64.0
J. D. B.	45.0	49.8	60.7
J. M.	38.7	49.8	59.4
E. S.	47.8	46.8	49.1
J. K.	36.2	45.5	55.0
H. G.	45.4	43.3	53.3

carbon dioxide capacity of whole blood at 40 mm. of CO_2 tension and 37.5°C.; and the carbon dioxide capacity of venous plasma determined according to the technique of Van Slyke (23). (For the last, venous blood was withdrawn under albolene, centrifuged in this condition, and the plasma removed to a separatory funnel and saturated with alveolar CO_2.) The observations are arranged according to the magnitude of the CO_2 content. The plasma capacity parallels the CO_2 content rather more closely than it does the CO_2 capacity of whole blood. But, if the CO_2 capacity

of the plasma is to be used as a measure of the bicarbonate content of the blood, it should vary directly as the CO_2 capacity of the whole blood.

The cardiac cases are not the only ones in which the plasma method fails to agree with the whole blood method. H. G. shows a slight acidosis on the basis of his plasma figures, although the whole blood CO_2 capacity is normal. In this case the venous carbon dioxide content is very low. The discrepancy is slight, but is quite striking in comparison with J. D. B., a cardiac patient with an absorption curve at the same level as that of H. G. Even in normal subjects estimations of the bicarbonates of whole blood from those of plasma may prove inaccurate. This is well illustrated in the case of D. P. B., who on several occasions showed a CO_2 capacity relatively higher in the plasma than in the whole blood.

In severe anemia the use of plasma may lead to misinterpretations. In this condition, as we shall show, the whole blood curve becomes relatively flat, approaching that of plasma. Changes in CO_2 tension, therefore, produce relatively small alterations in CO_2 content. The consequence is that the values obtained from whole blood are high in comparison with those obtained from plasma. This is especially noticeable in Cases A. B. and E. S. (The corresponding hemoglobin values were 32 and 35 per cent respectively.) This may explain the fact that Kahn and Barsky (24), studying the plasma in pernicious anemia, found a reduction of the alkali reserve in three cases, while the four cases in our series gave consistently high values for the CO_2-combining capacity of whole blood.

Finally, a further source of error lies in the fact that the absorption curves of plasma from different specimens of blood may differ. This has been demonstrated by Straub and Meier (22).

The use of the CO_2-combining power of the plasma as a measure of the alkali reserve will cause little or no error in most cases if it is employed only in those conditions in which the respiratory and circulatory systems are undisturbed and react in the normal manner to the natural stimuli. It was to subjects of this type that Van Slyke and coworkers (25) first applied it with such success that his method has become the standard method for the measurement of the alkali reserve. Henderson and Haggard

(26) have contended that whole blood is preferable to plasma. The latter, however, was chosen by Van Slyke with full recognition of the error involved, because of technical reasons, especially the fact that whole blood demanded immediate analysis, whereas plasma could be kept for a considerable length of time without deterioration. Furthermore, the coagulum produced by the addition of acid to whole blood rendered the cleansing of his pipette difficult. With the substitution of tartaric acid for sulfuric in the Van Slyke method one of the technical reasons for preferring plasma has been removed. The impossibility of keeping whole blood for any length of time remains an objectionable feature.

If, for this reason, the use of plasma is found necessary, variations in carbon dioxide saturation, the chief cause of error in the use of plasma, must be avoided. This can easily be effected by bringing the whole blood into equilibrium with a standard air-CO_2 mixture, *e.g.* alveolar air, before removing the plasma. If this were done at least the chief objection to the use of plasma would be removed. However, the air-CO_2 mixture used for saturation of whole blood must be more carefully standardized than is the case when plasma is employed. This is evident from the differences in the absorption curves of whole blood and plasma. Small variations in carbon dioxide tension have a much greater effect on the carbon dioxide content of whole blood. The use of alveolar air, which is the usual procedure with plasma might, with whole blood, introduce a perceptible error. Under resting conditions the alveolar CO_2 tensions of normal individuals should not vary by more than a few millimeters. As the corresponding change in the carbon dioxide content of the blood is only half as great, the use of alveolar air by an operator trained in respiratory methods should produce no serious errors.

Under these conditions plasma should give values that are dependable as far as the ability of the blood to neutralize acids other than carbonic is concerned. For the study of the respiratory function of the blood, on the other hand, whole blood alone can be used. In order to avoid unnecessary complications, the normal limits of variation at room temperatures will have to be more definitely established, as whole blood work up to the present has been confined almost entirely to physiological studies and saturation has been effected at body temperature.

Just as this paper was approaching completion the final report of the work of Joffe and Poulton (20) on the absorption curves of whole blood and plasma appeared. On the basis of their findings they also conclude that the carbon dioxide capacity of plasma separated from venous blood is not a proper measure of the available alkali of the blood. They advise a method identical with that here proposed.

Up to the time that Van Slyke published his method the presence of a carbon dioxide retention had not yet been demonstrated in any pathological condition, and, although the possibility of such an occurrence was clearly recognized by him, it did not seem of sufficient practical importance to outweigh the obvious technical advantages of plasma over whole blood. As applied to the study of diabetes the use of plasma probably introduced no serious error. When we presented our data to Dr. Van Slyke we found that he had come to practically the same conclusion as a result of some studies on anesthesia.

Carbon Dioxide Content of the Venous Blood in Cardiac Dyspnea.

It is clear that a discrepancy between the CO_2 capacity of whole blood and that of venous plasma may be produced by at least three factors: (1) An abnormal venous carbon dioxide tension; (2) a disturbance of the normal proportion of cells to plasma; (3) an abnormality in the carbon dioxide absorption curve of the plasma itself. The second of these we may assume to be unimportant in cardiac dyspnea, although we have not investigated the matter carefully. In the majority of cases hemoglobin determinations were made by means of the oxygen capacity and revealed no striking changes. The plasma absorption curve has not been determined. We have, however, studied the venous carbon dioxide content of a number of subjects.

The determination of the presence or absence of carbon dioxide retention in the venous blood is not so simple a matter as it may appear. The results obtained by previous observers are not at all consistent. Harrop (16) found that the difference between arterial and venous carbon dioxide content was sometimes increased in cardiac dyspnea, indicating a relative retention of carbon dioxide in the venous blood. Table IV gives a summary of Harrop's findings. The results obtained from the five cases

in our series appear in Table III. The two sets of observations are in essential agreement. The difference between arterial and venous blood was much more variable in decompensated cardiac cases than in normal subjects, and in some of the former was relatively increased. Scott (27) has recently reported the CO_2 content of the plasma of both arterial and venous blood lower than normal in cardiac decompensation. A few unpublished determinations made by one of us on venous plasma in 1916 were not in agreement with his findings. The values obtained were more variable than those obtained from normal subjects, but the variations were not predominantly low. In some cases they were quite high. The same is true of the values obtained from whole blood. Their only characteristic is a great range of variation (see Tables I and III). By comparison with Table V it will be seen that these variations bear a rough relation to the height of the absorption curves.

Neither Harrop's nor Scott's method can throw much light on the question of the presence or absence of a carbon dioxide retention. The absolute value for carbon dioxide content of either whole blood or plasma, whether arterial or venous, although of interest, cannot be accepted as a satisfactory criterion. The CO_2 content which is normal for a blood with a high absorption curve is excessive for a blood with a low absorption curve, if the reaction of the blood is to be maintained constant. The locus of points of equal pH is represented by straight lines passing through the origin. As the curves become lower, therefore, equal amounts of CO_2 have a greater effect in changing the pH. The presence of a carbon dioxide retention can only be determined, then, by a comparison of the carbon dioxide content of the blood with the height of the absorption curve. Such a comparison appears in the venous pH values in Table III. In the case of the three cardiac patients with low absorption curves (Cases 10, 11, and 12) there is a definite carbon dioxide retention which explains the falsely high values for bicarbonate obtained by the plasma CO_2 capacity method of Van Slyke (23).

SUMMARY AND CONCLUSIONS.

1. A study has been made of the carbon dioxide absorption curve of the blood of seven patients with cardiac decompensation

and dyspnea. In three out of the seven low curves were found. In two of these cases the curves returned to the normal level when compensation was reestablished. Clinically these patients were distinguished by the presence of an extreme degree of cyanosis with little dyspnea. No relation could be established between the reduction of the absorption curve and the oxygen unsaturation of the arterial or venous blood. A low absorption curve was not an indication of the severity of the condition. No signs of renal involvement were discovered in any of these cases.

2. The reduction of the alkali reserve indicated by these low absorption curves was not reflected in the plasma. The CO_2 capacity of the venous plasma is determined by the carbon dioxide tension at which it existed in the body rather than by the carbon dioxide-combining capacity of the blood. For this reason the determination of the plasma bicarbonates by the technique originally proposed is not applicable as a measure of the alkali reserve in conditions associated with CO_2 accumulation in the venous blood. A true measure of the bicarbonate content of blood is obtained from the carbon dioxide capacity of whole blood. As whole blood deteriorates when kept, while plasma does not, it may still be necessary to employ plasma for purposes of routine procedure. In this case errors due to variations in carbon dioxide saturation may be avoided by saturating the whole blood with a standard air-CO_2 mixture before separating the plasma. With this modification the method may be rendered applicable to the determination of the alkali reserve of the blood. For the study of the respiratory function of the blood, however, only whole blood should be used.

3. (*a*) In four cases arterial and venous carbon dioxide tension were determined. The alveolar carbon dioxide tension was also determined. The only consistent and characteristic finding was an increase in the difference between alveolar and arterial CO_2 tension. This difference varied from 13 to 19 mm.

(*b*) In two cases there was a definite CO_2 retention in the arterial and venous blood with a consequent lowering of the pH.

(*c*) No consistent increase in the difference between arterial and venous CO_2 content or tension could be demonstrated, although in one or two instances it was slightly greater than normal.

4. The causes of the discrepancy between the alveolar carbon dioxide tension and the carbon dioxide-combining capacity of the venous plasma in cardiac dyspnea are:

(*a*) The fact that the carbon dioxide capacity of the plasma gives values that are too high in the presence of carbon dioxide retention such as is sometimes found in cardiac dyspnea.

(*b*) The alveolar carbon dioxide is very low in proportion to the carbon dioxide content of the venous blood and does not bear a definite relation to the height of the absorption curve.

5. The causes of cardiac dyspnea seem to be: The fact that a greater ventilation is necessary to effect the normal carbon dioxide elimination. This is largely brought about by an impairment of the efficiency of the pulmonary mechanism for the exchange of gases between the blood and the outside air. This necessitates the maintenance of a greater difference in carbon dioxide pressure between the blood in the pulmonary circulation and the alveolar air in order to effect the normal carbon dioxide output. To maintain the carbon dioxide tension and the hydrogen ion concentration at the proper level the alveolar carbon dioxide tension must be abnormally low. In some cases a diminution of the circulation rate may be an additional factor in the production of a carbon dioxide acidosis. Finally in a certain proportion of the cases, at least, there is a real reduction of the available alkali of the blood. In at least two cases with a reduction of the available alkali of the blood were found indications of a comparative insensibility of the respiratory center to its natural physicochemical stimulus, the hydrogen ion concentration of the blood.

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EXPLANATION OF CHARTS.

Chart 7.

Case 7. J. D. B. Syphilitic aortitis. Dilatation of ascending aorta. Aortic insufficiency. Male, age 50 years, single, pastry cook.

Looks chronically ill. Moderate dyspnea even while at rest, but able to walk around ward. Faint cyanosis of lips and finger-tips. Lungs clear.

Heart very much enlarged, with loud systolic and diastolic murmurs, maximum over the aortic area. X-ray revealed aneurysmal dilatation of the ascending aorta.

Wassermann ++++.

Patient improved but slightly and is still in the hospital at the time of publication.

Experiment 1. Mar. 19, 1920.

Alveolar CO_2 before venous puncture, *mm*			25.5
			25.0
" CO_2 after " " "			25.2
CO_2 absorption curve:			
CO_2 tension, *mm. Hg*	77.2	47.7	23.8
CO_2, *vol. per cent*	62.9	48.7	37.1
	59.7		36.5
CO_2 content of venous blood, *vol. per cent*			49.8
CO_2 capacity of venous plasma (41.7 mm. Hg) *vol. per cent*			60.7
O_2 capacity of blood, *vol. per cent*			16.4

Experiment 2. Apr. 30. Condition practically unchanged, although cyanosis may be slightly increased.

Alveolar CO_2 before arterial puncture, *mm*				27.3
				27.1
				27.7
" CO_2 after " " "				27.6
CO_2 absorption curve:				
CO_2 tension, *mm. Hg*	59.8	47.2	28.4	
CO_2, *vol. per cent*	55.6	52.9	39.8	
	56.1	50.7	40.3	
CO_2 content of arterial blood, *vol. per cent*				49.3
				48.9
CO_2 " " venous " " " "				52.0
				53.4
Difference between arterial and venous CO_2, *vol. per cent*				3.6
O_2 capacity of blood, *vol. per cent*				13.9
O_2 content of arterial blood, *vol. per cent*				14.3
O_2 " " venous " " " "				3.7
O_2 consumption, *vol. per cent*				10.6
O_2 saturation of arterial blood, *per cent*				100
O_2 " " venous " " "				27
Respiratory quotient of blood				0.34

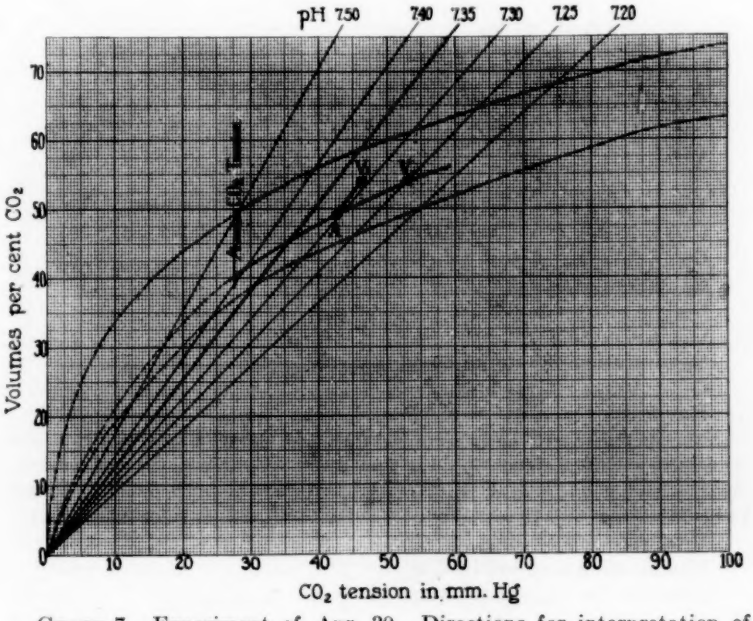

CHART 7. Experiment of Apr. 30. Directions for interpretation of charts are given in Paper I.

CHART 10.

Case 10. J. M. Mitral stenosis. Male, age 60 years, single, engineer. Looks chronically ill. Lies quietly in bed, without dyspnea or orthopnea. Very marked cyanosis of face and extremities. Veins of neck engorged, but not visibly pulsating. Brawny, pitting edema of feet and legs. Signs of moderate amount of fluid in the right pleural cavity. Liver very much enlarged and pulsating.

Temperature normal. Pulse absolutely irregular; rate 72 to 100. Blood pressure: systolic 138; diastolic 86. Heart moderately enlarged both to the right and left. Loud blowing, systolic murmur at apex, accompanied by rough thrill. No murmurs at base. Electrocardiogram shows auricular fibrillation.

Urine contains considerable albumin, with a few granular and hyaline casts. Specific gravity 1.023 to 1.027. Non-protein nitrogen of blood 43 mg. per 100 cc. Phenolsulfonephthalein excretion 60 per cent in 2 hrs. Discharged from hospital, improved, 1 week after last experiment.

Experiment 1. Apr. 8, 1920.

Minute volume of respirations, *cc.*	8,652
Tidal air 475 cc. Respirations per min.	18.2
CO_2 in expired air 2.40 per cent. CO_2 output per min., *cc.*	208
Vital capacity of the lungs, *cc.*	1,197

Experiment 2. Apr. 9. Condition unchanged.

Alveolar CO_2 before arterial puncture, *mm.*	25.0		
CO_2 absorption curve:			
CO_2 tension, *mm. Hg*	75.8	54.4	44.4
CO_2, *vol. per cent*	56.6	46.0	41.7
CO_2 content of arterial blood, *vol. per cent*	39.6		
	39.6		
CO_2 " " venous " " " "	49.5		
	50.0		
Difference between arterial and venous CO_2, *vol. per cent.*	10.2		
CO_2 capacity of venous plasma (40.9 mm. Hg), *vol. per cent.*	59.4		
O_2 " " blood, *vol. per cent*	20.7		
O_2 content of arterial blood, *vol. per cent*	16.7		
O_2 " " venous " " " "	8.5		
O_2 consumption, *vol. per cent*	8.2		
O_2 saturation of arterial blood, *per cent*	83		
O_2 " " venous " " "	42		
Respiratory quotient of blood	1.24		

Experiment 3. Apr. 23. Condition greatly improved. Quite comfortable. No edema. Signs of fluid in chest gone. Cyanosis still well marked. Heart action good, rate about 60, with practically no pulse deficit. Is able to be out of bed and to walk about ward without difficulty or discomfort.

Vital capacity of lungs, *cc.*	2,463
Minute volume of respirations, *cc.*	9,854
Tidal air 498 cc. Respirations per min.	19.8

CO_2 in expired air 2.72 per cent. CO_2 output per min., *cc.*		268
Alveolar CO_2 before arterial puncture, *mm.*		34.2
		35.3
" CO_2 after " " "		31.2
CO_2 absorption curve:		
CO_2 tension, *mm. Hg*	80.4	26.6
CO_2, *vol. per cent*	64.6	38.0
	63.9	40.5
CO_2 content of arterial blood, *vol. per cent*		53.8
		54.2
CO_2 " " venous " " " "		63.0
		63.7
Difference between arterial and venous CO_2, *vol. per cent.*		9.3
CO_2 capacity of venous plasma (52.0 mm. Hg), *vol. per cent.*		75.7
O_2 " " blood, *vol. per cent*		20.8
O_2 content of arterial blood, *vol. per cent*		22.0
O_2 " " venous " " " "		11.1
		11.0
O_2 consumption, *vol. per cent*		10.9 or 9.7
O_2 saturation of arterial blood, *per cent*		100
O_2 " " venous " " "		53
Respiratory quotient of blood		0.85 or 0.96

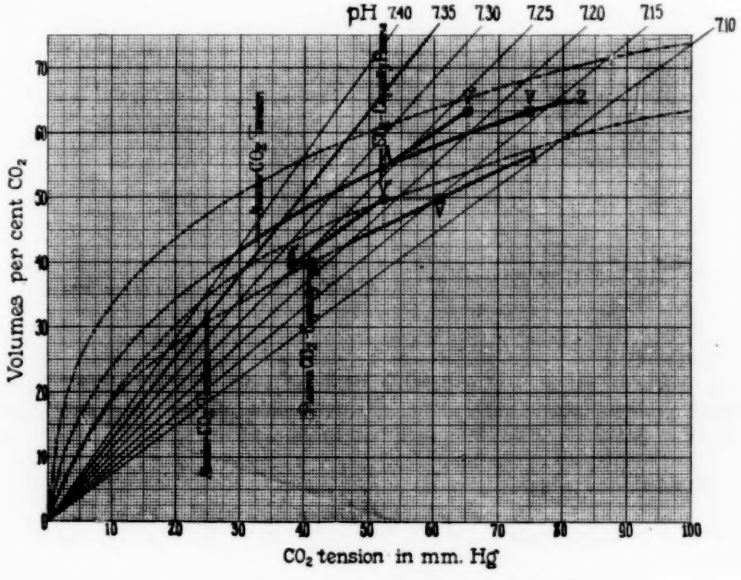

CHART 10. 1. Experiment of Apr. 9.
2. Experiment of Apr. 23.

CHART 11.

Case 11. J. K. Mitral stenosis. Male, age 35 years, single, elevator operator. Looks chronically ill. Lies quietly in bed with only slight dyspnea and orthopnea. Face and extremities are a livid purple. Slight, pitting edema of feet, ankles, and legs. Few sibilant and sonorous râles scattered throughout lungs, but no signs of pleural effusion. Liver enlarged and pulsating. Temperature normal. Pulse totally irregular, radial rate 55, heart rate 92. Blood pressure: systolic 140, diastolic 100. Heart enlarged both to left and right. Loud systolic and soft diastolic murmurs, maximum over apex. Pulmonic second sound accentuated. Electrocardiograms show auricular fibrillation.

Urine shows cloud of albumin, no casts. Specific gravity 1.018.

Discharged from hospital improved, 9 days after last experiment.

Experiment 1. Apr. 16, 1920.

Alveolar CO_2 before arterial puncture, *mm*			23.0
			22.1
" CO_2 after " " "			21.4
			21.4
CO_2 absorption curve:			
CO_2 tension, *mm. Hg*	68.0	47.1	36.7
CO_2, *vol. per cent*	47.7	38.3	35.8
	47.4	39.4	
CO_2 content of arterial blood, *vol. per cent*			36.8
			35.0
CO_2 " " venous " " " "			44.8
			46.1
Difference between arterial and venous CO_2, *vol. per cent*			9.6
CO_2 capacity of venous plasma (37.8 mm. Hg), *vol. per cent.*			55.0
O_2 " " blood, *vol. per cent*			26.3
			26.7
O_2 content of arterial blood, *vol. per cent*			24.2
O_2 " " venous " " " "			9.9
O_2 consumption of " " " "			14.3
O_2 saturation of arterial blood, *per cent*			91
O_2 " " venous " " "			37
Respiratory quotient of blood			0.67

Experiment 2. Apr. 20. Greatly improved. No dyspnea. Cyanosis less marked, but still considerable. Edema gone. Lungs clear. Pulse good quality. Heart rate about 40. No pulse deficit (digitalis heart block). Arthritis of left foot with temperature of 102°F.

Vital capacity of the lungs, *cc*	1,800
Alveolar CO_2 before arterial puncture, *mm*	30.2
	34.7
" CO_2 after " " "	32.5

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	37.8
CO_2, *vol. per cent*	49.1
	50.7
CO_2 content of arterial blood, *vol. per cent*	53.7
	51.0
O_2 " " " " " " "	25.0
O_2 " " venous " " " "	22.5
O_2 consumption, *vol. per cent*	2.5
O_2 saturation of arterial blood, *per cent*	94
O_2 " " venous " " "	85

(O_2 capacity of Apr. 16 used for calculations.)

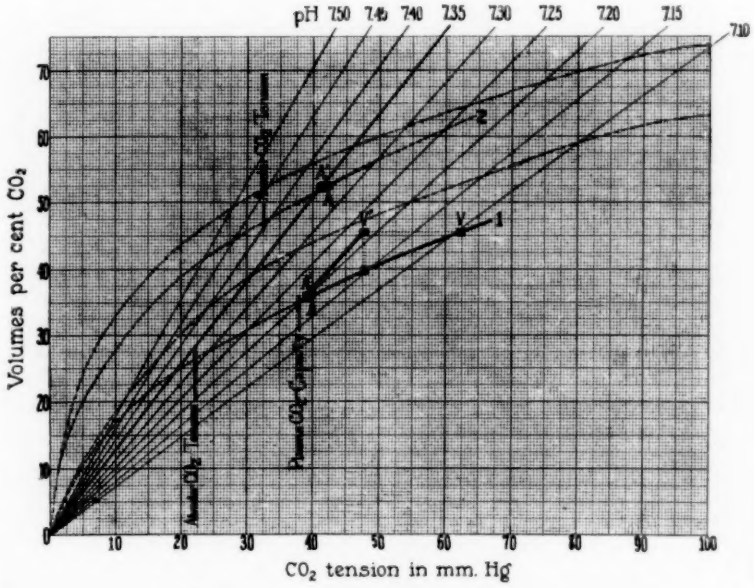

CHART 11. 1. Experiment of Apr. 16.
2. Experiment of Apr. 20.

CHART 12.

Case 12. C. C. Mitral stenosis. Looks chronically ill. Lies quietly in bed, with only slight dyspnea and no orthopnea. Very marked cyanosis of head and extremities. Somewhat irrational.

Experiment 1.

Alveolar CO_2 before arterial puncture, *mm.*				28.0
				26.1
				27.8
" CO_2 after " " "				27.5
CO_2 absorption curve:				
CO_2 tension, *mm. Hg*	66.2	47.6	39.0	
CO_2, *vol. per cent*	43.7	34.8	33.0	
	45.3	35.9	33.0	
CO_2 content of arterial blood, *vol. per cent*				33.4
				33.6
CO_2 " " venous " " " "				43.1
				42.6
Difference between arterial and venous CO_2, *vol. per cent*				9.4
O_2 capacity of blood, *vol. per cent*				18.3
O_2 content of arterial blood, *vol. per cent*				18.0
O_2 " " venous " " " "				3.2
O_2 consumption, *vol. per cent*				14.8
O_2 saturation of arterial blood, *per cent*				98
O_2 " " venous " " "				18
Respiratory quotient of blood				0.64

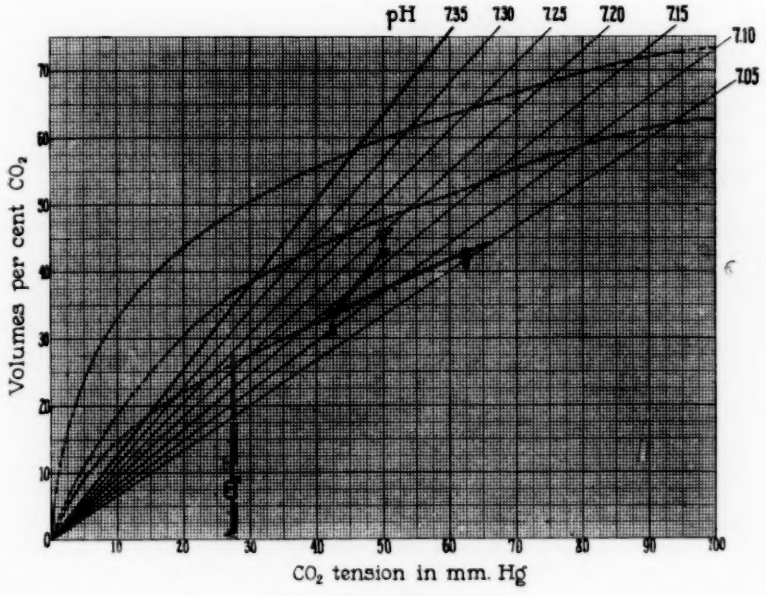
pH
7.35
7.30
7.25
7.20
7.15
7.10
7.05
Volumes per cent CO_2
CO_2 tension in mm. Hg

CHART 12.

CHART 13.

Case 13. M. C. Bronchial asthma with severe emphysema. Male, age 40 years, single, hospital orderly.

Complains of severe dyspnea and cough. Has been admitted to the hospital several times. Looks acutely distressed. Is sitting up in bed. Respirations are rapid and labored, with prolonged, wheezing expiration. Most intense cyanosis of head and extremities, which are a deep purple. Veins of neck engorged. Lungs show boardy resonance everywhere. Expiratory breath sounds prolonged, high pitched, and wheezing. Rhonchi and râles of all kinds heard all over chest. Heart: apex and borders of dulness not made out. Sounds distant. No murmurs made out. Pulse about 100.

Experiment 1. May 12, 1920.

Alveolar CO_2 before arterial puncture, *mm*.				39.8
				44.1
				41.2
" CO_2 after " " "				40.9
CO_2 absorption curve:				
CO_2 tension, *mm. Hg*.	61.9	15.1	75.1	39.6
CO_2, *vol. per cent*.	57.0	32.2	56.9	48.7
	56.3	31.0	58.2	50.1
CO_2 content of arterial blood, *vol. per cent*.				58.4
				56.7
CO_2 " " venous " " " " "				63.9
				62.5
Difference between arterial and venous CO_2, *vol. per cent*.				5.7
CO_2 capacity of venous plasma (51.2 mm. Hg), *vol. per cent*.				74.5
O_2 " " blood, *vol. per cent*.				21.3
O_2 content of arterial blood, *vol. per cent*.				17.2
O_2 " " venous " " " " "				11.0
O_2 consumption, *vol. per cent*.				6.2
O_2 saturation of arterial blood, *per cent*.				81
O_2 " " venous " " "				52
Respiratory quotient of blood.				0.92

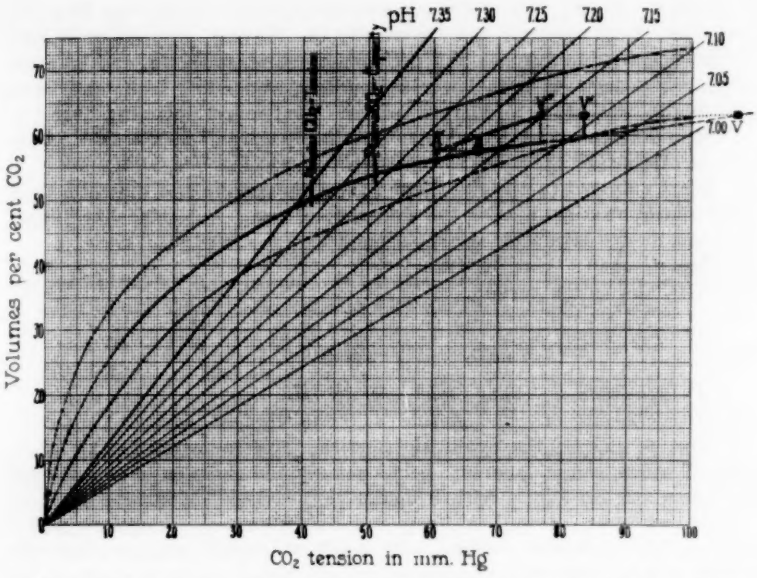

CHART 13. V″ represents the venous CO_2 tension corrected for oyxgen unsaturation, using the value of 0.414 for K (see text).

Additional Protocols.

Case 6. G. B. Paroxysmal tachycardia. Male, age 32 years, single, cook. Acutely ill. Some dyspnea, orthopnea, and cyanosis. Edema of both ankles. Signs of moderate amount of fluid in the right pleural cavity and in the abdomen. Liver enlarged. Temperature about 103° F. Pulse regular, rate about 180. Heart enlarged in both directions, but without signs of a valvular lesion.

Condition did not respond to treatment and patient died suddenly 6 days after the experiment here reported.

Experiment 1. Mar. 24, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	78.7	47.2	11.6
CO_2, *vol. per cent*	61.1	49.1	27.4
			26.4

CO_2 content of venous blood, *vol. per cent*	54.8
O_2 capacity of blood, *vol. per cent*	21.7
O_2 content of venous blood, *vol. per cent*	5.0
O_2 saturation of venous blood, *per cent*	23

Case 8. E. H. Mitral stenosis and insufficiency. Male, age 39 years, butcher. Looks acutely and chronically ill. Sitting, propped up in bed, with considerable dyspnea and orthopnea, pallid, cyanotic, and in acute distress. Edema of both lower extremities, abdominal wall, and back. Double hydrothorax. Moderate ascites. Very large, pulsating liver.

Temperature about 100. Pulse absolutely irregular, rate slow as the result of digitalis therapy.

Heart enlarged to the right and left, with systolic and diastolic thrill and shock, maximum over the midcardiac area. Auricular fibrillation. Patient died 2 days after the experiment here reported.

Experiment 1. Mar. 10, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	16.0	34.8	69.5
CO_2, *vol. per cent*	19.0	46.2	59.2
			61.1

CO_2 content of venous blood, *vol. per cent* 51.1

CO_2 capacity of " plasma (44.0 mm. Hg) *vol. per cent*... 64.0

Case 9. P. O. S. Syphilitic aortitis. Aortic insufficiency. Male, age 49 years, single, laborer. Looks acutely and chronically ill. Marked dyspnea and orthopnea with irregular respirations. Moderate cyanosis. Edema of lower extremities, back, and abdominal wall. Signs of fluid in both pleural cavities. Liver very much enlarged. Temperature 100. Pulse regular, rate 90, collapsing in type. Blood pressure: systolic 140, diastolic 50. Heart very much enlarged, especially downward and to the left. Soft, systolic and loud, buzzing, diastolic murmurs, maximum over the base of the heart. Diastolic murmur accompanied by a thrill.

Wassermann ++++.

Patient died 3 weeks after the last experiment without having left the hospital.

Experiment 1. Mar. 9, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	66.8	59.9	20.6
CO_2, *vol. per cent*	63.9	60.7	39.0
	65.4		39.7

CO_2 content of venous blood, *vol. per cent* 62.1

CO_2 capacity of venous plasma (51.1 mm. Hg), *vol. per cent*.. 74.2

Experiment 2. Mar. 23. Continues to grow worse. Hydrothorax increasing. Irrational. Dyspnea is less, possibly because of morphine, but respirations are more irregular. Considerable cyanosis.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	(26.4)	93.7	51.8
CO_2, *vol. per cent*	42.8	65.8	52.5
	42.8		

CO_2 content of venous blood, *vol. per cent* **56.9**

O_2 capacity of blood, *vol. per cent* 22.3

III. THE CARBON DIOXIDE ABSORPTION CURVE AND CARBON DIOXIDE TENSION OF THE BLOOD IN SEVERE ANEMIA.

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Carbon Dioxide Absorption Curve in Severe Anemia.

An analysis of the CO_2 absorption curve of normal blood has been made in Paper I of this series (1). In the course of the investigation we had occasion to study the blood of a case of pernicious anemia and found such marked variation from the normal in the shape and character of the absorption curve that we were stimulated to study the bloods of other cases of anemia as they presented themselves in the wards. We later found that the CO_2 absorption curves from the blood of one case of pernicious anemia and from one case of moderate secondary anemia had been constructed in 1917 by Hasselbalch (2) who used them as collateral evidence in his argument concerning the true nature of the supposed acidosis of newly born children. In this paper we wish to present the absorption curves of several patients with varying degrees of anemia, to discuss the factors which may cause the curve of anemic blood to differ from the normal, and finally to show the bearing of the findings upon the respiratory mechanism and particularly upon the dyspnea of severe anemia.

The methods employed have been described in detail in Paper I. The absorption curve of fully oxygenated blood and the CO_2 content of venous blood have been determined. Upon three of the patients arterial puncture was performed and the CO_2 content of arterial and of venous blood was ascertained. The percentage of hemoglobin was obtained in all cases by the oxygen capacity method of Van Slyke (3). In one case the alveolar CO_2 tension was also determined.

TABLE I.

Subject.	Date.	Tension of CO_2. (1)	CO_2 absorbed. (2)	Venous CO_2 content. (3)	Arterial CO_2 content. (4)	CO_2 capacity of plasma. (5)	Venous oxygen content. (6)	Arterial oxygen content. (7)	Oxygen capacity. (8)	Hemoglobin. Haldane scale. (9)	Diagnosis and remarks. (10)
	1920	*mm. Hg*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*mm. Hg*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*per cent*	
Jas. M.	June 10	0.1	45.60	61.28	57.93		0.54	2.92	3.41	18.9	Probable pernicious anemia, male, age 62. Red blood count 710,000. Pulse 88–120. Respirations 18–28. Temperature 97–99°.
			45.60	61.08	57.34				3.28	17.7	
		39.8	58.12								
			59.66								
		78.4	67.38								
			65.90								
C. P.	May 28	24.0	47.30	55.18	52.24		1.55	4.37	4.34	23.1	Pernicious anemia, male, age 55. Red blood count 1,000,000. Pulse 108–112. Respirations 24–28. Temperature 100–101°.
			48.34	54.50	51.55						
		46.8	55.59								
			55.87								
		93.1	63.00								
			64.60								
R. H.	June 10	30.2	46.00	52.08	50.87		0.00	4.76	4.36	23.5	Gastric carcinoma, female, age 44. Red blood count 2,300,000. Pulse 80–104. Respirations 20–32. Temperature 99–102°.
		47.9	51.90	53.43	50.47						
			55.12								
		82.2	60.13								
			61.27								

A. B.	Mar. 16	18.0	51.10	63.99		47.54			6.17	33.3	Pernicious anemia, female, age 47. Red blood count 1,500,000–1,000,000. Pulse 120–128. Respirations 20–24. Temperature 98–102°.
		46.3	64.60	63.91					5.76	31.1	
			60.89								
		80.4	70.00								
	Apr. 1	20.6	48.35						4.80	25.90	
		52.2	58.10								
		91.0	65.10								
E. S.	Mar. 3	6.4	33.52	46.77		33.78	3.66		6.90	37.4	Chronic nephritis, hypertension, and uremia, female, age 22. Red blood count 4,060,000. Pulse 84–108. Respirations 20–28. Temperature 98–99.2°. Blood pressure: systolic 190, diastolic 110.
			33.15						6.45	35.4	
		30.5	44.80								
		53.6	50.67								
			52.08								
C. A.	Mar. 22	25.8	61.40						9.88	53.3	Gastric carcinoma, female.
		38.8	69.80								
		61.0	74.60								
N. B.	Mar. 15	7.1	31.60	60.13		45.26			10.08	54.7	Pernicious anemia, female, age 53. Red blood count 2,700,000. Pulse 92. Temperature 98–103°.
			32.27			45.47			9.61	52.2	
		42.2	53.81								
			52.95								
		71.9	63.46								
			63.18								

The cases upon which this study is based include three patients with pernicious anemia, one with a severe anemia probably pernicious, two suffering from gastric carcinoma, and one from chronic nephritis. A protocol of the combined data of all observations is given in Table I. In Column 1 are included the tensions of CO_2 to which the blood was exposed and in Column 2 the volume of CO_2 absorbed at each tension is given. From these data the CO_2 absorption curves were constructed. The other columns of the table are self-explanatory.

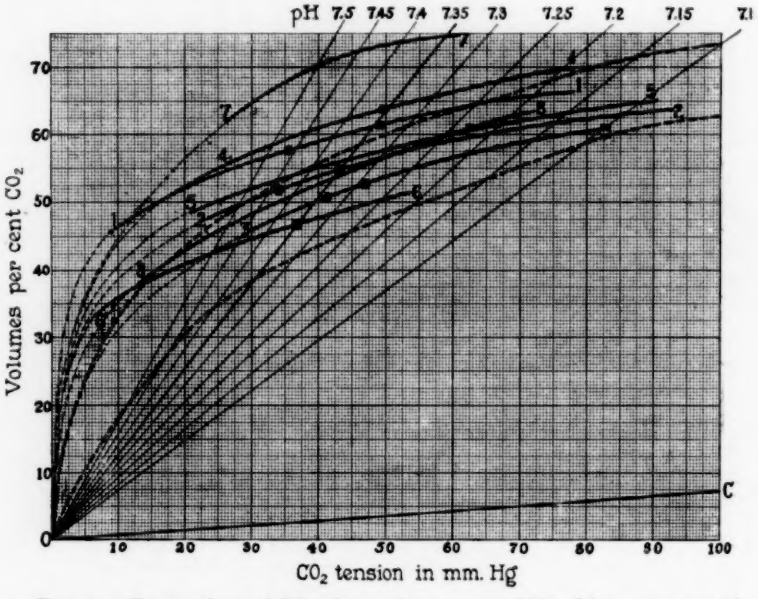

FIG. A. Comparison of CO_2 absorption curves of blood in severe anemia with curves of normal blood.

Solid curved lines: curves from blood of anemia. 1. Jas. M., 18 per cent hemoglobin. 2. C. P., 23 per cent hemoglobin. 3. R. H., 23 per cent hemoglobin. 4. A. B., Mar. 16, 1920, 32 per cent hemoglobin. 5. A. B., Apr. 1, 1920, 26 per cent hemoglobin. 6. E. S., 36 per cent hemoglobin. 7. C. A., 53 per cent hemoglobin. 8. N. B., 53 per cent hemoglobin.

Broken curved lines: limits of dissociation curves from blood of normal persons.

Straight diagonal line *OC* represents the CO_2 in solution and in form of H_2CO_3.

Other diagonal lines represent pH of blood.

● = Arterial CO_2 content.

■ = Venous CO_2 content.

The absorption curves are represented in Fig. A. The solid curved lines are curves of blood from patients with anemia. Each of these is numbered and the numbers, the initials of the patients to which they refer, and the percentage of hemoglobin contained in the blood are given in the legend. The curved broken lines represent the upper and lower limits of the absorption curve for the blood of normal individuals (1). The diagonal, *OC*, gives the amount of CO_2 in physical solution and in the form of carbonic acid for each tension. The other diagonal lines indicate the hydrogen ion concentration in terms of the negative logarithm, pH, of Sörensen (4).

From a superficial survey of the figure it will be seen that the curves of blood in anemia differ markedly from the normal. They are more nearly parallel to the base line. The volume of CO_2 absorbed at low tensions is relatively large while the volume at high tensions is less than normal. In these respects our results are entirely in keeping with those of Hasselbalch (2). The curve of blood from severe anemia resembles the curve of plasma quite as closely as it does that of normal whole blood.

In Fig. B the CO_2 absorption curves of 0.03 M sodium carbonate solution, of 0.1 M sodium phosphate solution, of plasma, and of normal blood have been drawn for the purpose of comparing their slopes with that of the blood of Jas. M., a patient with severe anemia. The curves of phosphate solution and of plasma have been constructed from the figures given by Van Slyke and Cullen (5). The curve of carbonate solution is derived from the formula of Parsons (6).

The difference in the slope of the curves can be explained by the presence and character of the acids in the various solutions. In a solution of sodium carbonate where H_2CO_3 is the only acid, essentially all the alkali combines with CO_2 at low tensions. Below 10 mm. of CO_2 tension the curve is steep, while at all tensions above 10 mm. the curve is flat and parallels the line of physical absorption, *OC*. In plasma, in whole blood, and in the phosphate solutions, all of which contain both H_2CO_3 and other potential acids, the alkali is divided between the acids in proportions depending upon their relative strength and molecular concentration. The amount of alkali which may combine with carbonic acid between any two tensions, and hence the slope of

the CO_2 absorption curve, will depend upon the character and concentration of the other acid substances. For this reason it is important to consider the potential acids which exist in normal blood.

The plasma contains serum proteins which may have acid properties under conditions existing within the body. Attempts

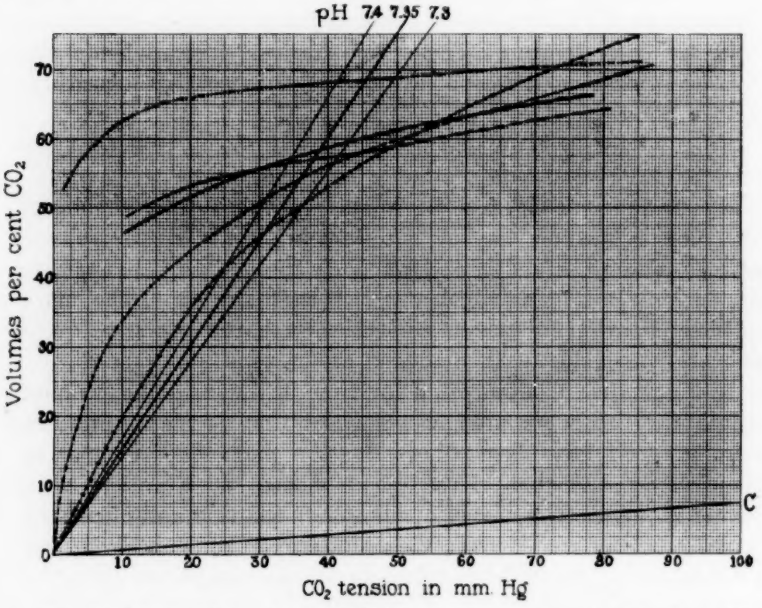

FIG. B. Comparison of curve of blood of severe anemia with other CO_2 absorption curves.

..... = 0.03 M Na_2CO_3 solution.
--x--x--x-- = 0.1 M Na_2HPO_4 solution.
—·—·—·— = Plasma.
---------- = Normal blood.
———— = Blood of Jas. M.; severe anemia.

to measure the magnitude of their acid action have given rise to conflicting results (7–10). The close resemblance between the curve of plasma and that of bicarbonate indicates that their influence is not great under normal conditions. Within the red blood cells there are two substances of potentially acid character, the phosphates and hemoglobin. The influence of the former

cannot be estimated quantitatively. It will be seen, however, that the curve of phosphate solution resembles closely that of normal whole blood. The presence of phosphates might from theory play a considerable rôle in the production of the typical blood curve (11). The action of hemoglobin as a potential acid has been emphasized recently by many investigators (12–15). It has been found to be an amphoteric substance which at the hydrogen ion concentrations existing within the body may have the properties of a weak acid.

In anemia the red blood cells are diminished in number and the reduction is accompanied by a decrease in the concentration of hemoglobin. If the action of hemoglobin as an acid is of importance, a marked reduction in its concentration should produce a flattening of the slope of the curve, which, in the most severe anemias, should resemble the curve of plasma. It has been seen that this actually occurs. The absorption of CO_2 by the blood of Jas. M. with 18 per cent hemoglobin occurs in a manner more like plasma than normal whole blood.

If hemoglobin were the only substance of importance, the slope of the curve should vary directly with the concentration of the pigment. Our results do not show this. The flattening of the curve is detectable only in severe anemia. Curves of blood containing 80 per cent of hemoglobin may have as steep a slope as those with 120 per cent (1). Careful measurements of the curves have failed to disclose any exact relation between the two phenomena. In this respect our curves are not in accord with those of Hasselbalch, who found the slope of the curve to vary directly with the percentage of hemoglobin. The most striking relation between the factors was apparent at low tensions, below those at which most of our curves were constructed. Hasselbalch's curves indicate that the blood of severe anemia does not lose all its CO_2 at 0 mm. of CO_2 tension. If this should be true, the extrapolations, the dotted portions of the curves in Fig. A, may be misleading.

In our data there is no exact relation between the percentage of hemoglobin and the slope of the absorption curve. Other factors must exert an influence. What these factors may be is, for the present, a matter for speculation only. In anemia plasma constitutes a large proportion of the total blood volume. Under

these circumstances the influence of the serum proteins upon the slope of the curve may vary in the different bloods studied. Straub and Meier (14) presented a plasma curve from a patient with polycythemia which differed from the normal and indeed resembled the curve of normal whole blood. It is possible that differences in color index, variations in the relation of the number of red blood cells to the percentage of hemoglobin, may exert some influence upon the shape of the curve. The curve of E. S. indicates that this factor is not of great importance. Although the blood of E. S. contains 4,000,000 red blood cells per cubic millimeter, the curve is as flat as that of the blood of A. B. with 1,500,000 red blood cells.

TABLE II.

Subject.	CO_2 absorbed at 40 mm. tension.
	vol. per cent
Jas. M.	58.4
R. H.	49.2
C. P.	53.0
A. B.	60.0
	54.2
E. S.*	47.0
C. A.	69.0
N. B.	51.5
Average	56.5
" of normal bloods	48.95

* E. S., a nephritic with edema and reduction of plasma bicarbonate. Omitted in calculation of average.

The slope of the curves of blood in anemia implies that at low CO_2 tensions the content of CO_2 will be relatively high. Furthermore, in anemia, plasma constitutes a large proportion of the total blood volume. Per unit volume, plasma contains more alkali than the red blood cells. This also will have the effect of making the CO_2 content of the blood higher than that of normal blood. That the curve in anemia, however, is not invariably high is shown by the curve of the blood of E. S., a chronic nephritic, whose alkali reserve, measured by the plasma method of Van Slyke, was diminished. The CO_2 content of the blood at the physiologically important tension of 40 mm. is shown in Table II.

The difference between the CO_2 content of arterial and venous blood in the three cases studied is much smaller than is usually observed in normal individuals.

Probably the chief physiological significance of the flat absorption curve of blood in anemia is the information which it gives concerning the combining power of blood between any two CO_2 tensions. A flat curve expresses diagrammatically the fact that the blood which it represents will combine with less CO_2 for a given rise in CO_2 tension and will lose less CO_2 for a given fall in tension.

The comparatively low combining power of blood in severe anemia between the tensions of 40 and 60 mm. is represented in a roughly quantitative way in Table III.

TABLE III.

Subject.	CO_2 content at 40 mm.	CO_2 content at 60 mm.	Difference.
	vol. per cent	*vol. per cent*	
Jas. M.	58.4	63.6	5.2
C. P.	53.0	58.8	5.8
R. H.	49.2	55.9	6.7
A. B.	60.0	66.0	6.0
	54.2	58.7	4.5
E. S.	47.0	51.7	4.7
C. A.	69.0	74.0	5.0
N. B.	51.5	58.4	6.9
Average difference			5.6
" normal difference			6.8

The decreased power of the blood in anemia to absorb or dissociate CO_2 with varying CO_2 tensions implies that changes in hydrogen ion concentration will be greater for each shift in tension. This is shown diagrammatically in Fig. A by the relation of the flat curves of anemia to the diagonal pH lines. The actual changes in hydrogen ion concentration between arterial and venous blood within the body are, however, influenced by other factors. The curves of blood in anemia are higher than the normal. This tends to lessen the effect of the flat curves upon hydrogen ion concentration, a fact indicated in the charts by the divergence of the pH lines as they pass from the origin. At 40 volumes per cent of CO_2 the pH 7.30 and 7.40 lines are

TABLE IV.

Results of the Study of Six Patients with Severe Anemia.

Subject.		Date.	Alveolar CO_2 tension.	Alveolar reaction.	Arterial CO_2 content.	Arterial CO_2 tension.		Arterial reaction.		Venous CO_2 content.	Venous CO_2 tension.		Venous reaction.		Difference between arterial and alveolar CO_2 tension.	Difference between arterial and venous CO_2 content.	Difference between arterial and venous CO_2 tension.	Difference between arterial and venous reaction.
						Uncorrected.	Corrected.	Uncorrected.	Corrected.		Uncorrected.	Corrected.	Uncorrected.	Corrected.				
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
		1920	*mm. Hg*	*pH*	*vol. per cent*	*mm. Hg*	*mm. Hg*	*pH*	*pH*	*vol. per cent*	*mm. Hg*	*mm. Hg*	*pH*	*pH*	*mm. Hg*	*vol. per cent*	*mm. Hg*	*pH*
18.	N. B.	Mar. 15								60.1	60.0		7.24					
19.	A. B.	" 16 Apr. 1								63.9	50.0		7.35					
14.	R. H.	June 10			50.7	41.0	41.0	7.34	7.34	52.8	47.0	43.0	7.30	7.33		4.8	2.0	0.01
15.	Jas. M.	May 5			57.6	35.0	35.0	7.47	7.47	61.2	49.0	45.0	7.35	7.38		3.9	10.0	0.09
17.	C. P.	" 28	28.2	7.50	51.9	33.5	33.5	7.44	7.44	54.9	42.5	41.0	7.35	7.37	5.3	3.0	6.5	0.07
16.	E. S.*	Mar. 3								46.8	45.0	41.5	7.28	7.32				
Maximum*					57.6	41.0	41.0	7.47	7.47	63.9	60.0	45.0	7.35	7.38		4.8	10.0	0.09
Minimum*					50.7	33.5	33.5	7.34	7.34	52.8	42.5	41.0	7.24	7.33		3.0	2.0	0.01

* The values obtained from the experiment on E. S., Case 16, have been omitted from the summary of maximum and minimum values because the anemia in this case was complicated by a severe chronic nephritis with uremia.

separated only about 4 mm. of CO_2 tension. At 60 volumes per cent this difference has increased to 10 mm. of CO_2 tension. This attempt at compensation is, however, counterbalanced by the reduction of the effect of oxygen on the curve. It will be remembered that the curves of Fig. A are those of blood containing oxygenated hemoglobin. The curve of reduced blood is always higher than that of the same blood fully oxygenated. Since the hemoglobin of venous blood is partially reduced, the venous points are not correctly represented on the curves of Fig. A, but should lie on a higher curve. If, as was indicated in Paper I of this series, the effect of oxygenation or reduction of the blood upon the height of the CO_2 absorption curve is a direct function of the hemoglobin, it is evident that the effect of oxygen unsaturation on blood from anemia will be less than on normal blood. The complete oxygen unsaturation of a blood with 25 per cent hemoglobin will increase the height of the absorption curve no more than will a 25 per cent unsaturation of a blood with 100 per cent hemoglobin. The net result of these various factors upon the changes in hydrogen ion concentration between arterial and venous blood is shown in Charts 14 to 17 and in Column 16 of Table IV. In spite of the small difference in carbon dioxide content between arterial and venous blood there is a considerable difference in pH, amounting in Cases 15 and 17 to 0.07. This is partly due to the diminished slope of the absorption curve, but even more to the loss of the oxygen effect. Even complete oxygen unsaturation cannot compensate a carbon dioxide change of more than 1 volume per cent in a subject with only 25 per cent hemoglobin.

Carbon Dioxide Tension and Hydrogen Ion Concentration of the Arterial and Venous Blood.

The hydrogen ion concentration of the arterial blood in the three cases studied lies at or above pH 7.34, in Case 15 (Chart 15) as high as 7.47. Means and coworkers (16) have also found the arterial pH quite high. In Case 17 (Chart 17) the alveolar carbon dioxide tension is correspondingly low, giving an alveolar pH of 7.50. In another case of anemia Means found the alveolar pH to be 7.43. This would indicate a tendency toward an arterial

alkalosis and a ventilation somewhat in excess of that which should be expected from the height of the absorption curve.

Although the arterial pH is in general rather high, the venous pH falls surprisingly close to the 7.35 line. In the four instances in which we have sufficient data to calculate the corrected venous pH the limits of variation are 7.32 to 7.38. In Means' case and our Case 19 it is also evident that the venous pH must be much closer to the average normal than is that of the arterial blood. Of course this may be due only to a peculiar coincidence, but it appears too frequently to pass without speculation.

It is generally stated that the respiratory center is controlled by the hydrogen ion concentration of the arterial blood and, in turn, responds in such a way as to maintain the arterial hydrogen ion concentration constant. It seems more probable, however, that the true controlling factor is the reaction of the tissue fluids in the respiratory center. Presumably this reaction is dependent upon carbon dioxide tension, just as it is in the blood. The carbon dioxide tension of the tissues must, however, always exceed that of the arterial blood and must, during the greater part of the circulation at least, be higher than that of the venous blood. In general it must be much closer to that of the venous blood. If the general tendency of the respiratory mechanism, then, is to maintain the pH of the tissue fluids in the respiratory center constant, one should expect the pH of the venous blood from the center to remain constant rather than that of the arterial blood which passes to the center.

This is not apparent in normal persons because, with a normal concentration of hemoglobin, the effect of oxygen on the carbon dioxide absorption curve renders the pH of arterial and venous blood practically identical.

DISCUSSION.

The influence of these factors upon the mechanism of respiration is apparent. The amount of CO_2 carried from the tissues for each change in tissue tension is less than normal. Unless the blood flow is increased, this will tend to result in accumulation of CO_2 within the tissues. With each increase in CO_2 tension, the hydrogen ion concentration rises with relative rapidity. Further-

more, the same difficulty which the blood has in absorbing CO_2 from the tissues is experienced in dissociating it in the lungs. For a given fall of tension the blood of anemia will dissociate less CO_2 than normal. Unless the ventilation is increased the CO_2 will tend to accumulate in the arterial blood and a vicious circle will be established. There is present in severe anemia a tendency to accumulation of CO_2 in the tissues, a diminished ability of the blood to lose CO_2 in the lungs, and a relatively rapid change in hydrogen ion concentration with any change in the CO_2 tension, all factors which tend to excite the respiratory center and produce dyspnea.

It is not surprising, therefore, to find that in severe anemia hyperpnea is present. It is probably not necessary to draw a causal relation between dyspnea and oxygen want even though the presence of the latter should be established. In regard to the question of oxygen consumption, however, the findings here presented suggest an interesting possibility. Barcroft (17) found that *in vitro* the last portions of oxygen were separated from hemoglobin with great difficulty. It is here shown in Cases 14 and 15 that the last bit of oxygen can be removed during the passage of the blood through the tissues. This has been shown previously by Lundsgaard (18) and others. Barcroft also found that the last portions of oxygen were more readily given off if the hydrogen ion concentration of the solution was increased. It seems possible that the more rapidly changing hydrogen ion concentration of the blood of severe anemia may explain why the last portions of oxygen can be freed during the comparatively brief interval of the passage of the blood through the tissues.

CONCLUSIONS.

1. The CO_2 absorption curves of blood from six patients with severe anemia have been studied. They have been found to have a flatter slope than have the curves of normal blood.

2. This is explained in part by the low percentage of hemoglobin. The flatness of the curves, however, is not exactly proportional to the diminution in the percentage of hemoglobin.

3. The blood of severe anemia has a diminished power of absorbing or dissociating CO_2 with changes of CO_2 tension.

Absorption of CO_2 from the tissues or loss of CO_2 from the lungs is probably accomplished with difficulty.

4. In three cases the hydrogen ion concentration of the arterial blood was determined and found to be relatively low, the pH being as much as 7.47 in one instance.

5. The venous pH was surprisingly constant, varying in four cases between 7.32 and 7.38.

6. In spite of the relatively small difference in carbon dioxide content between arterial and venous blood, the corresponding difference in pH is considerable, reaching 0.07 in two cases. This is due to the flat absorption curve and the loss of the compensating effect of oxygen unsaturation.

7. In severe anemia changes in CO_2 tension produce relatively small changes in CO_2 content and relatively great variations in hydrogen ion concentration. The fault in the CO_2-carrying power and the greater changes in hydrogen ion concentration may explain the dyspnea of anemia.

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CHARTS

EXPLANATION OF CHARTS.

CHART 14.

Case 14. R. H. Gastric carcinoma with severe anemia. Female, age 44 years, married. Looks chronically ill. Somewhat irrational. Well nourished, but shows some evidence of loss of weight. Skin pale and yellowish. Lips pale, with slight cyanotic tinge; similar cyanotic tinge in finger-nails. No signs of dyspnea or hyperpnea while at rest in bed.

Lungs show few, crackling râles at bases, especially on left side. Heart not enlarged. Blowing, systolic murmur and soft, diastolic murmur, maximum over aortic area. Pulse soft, regular, rate about 90. Blood pressure: systolic 150, diastolic 40. X-ray examination showed a small effusion in left pleural cavity. Thoracentesis obtained a small amount of viscid, bloody fluid.

Urine showed occasional trace of albumin, no casts. Specific gravity 1.010 to 1.018.

Non-protein nitrogen of blood 43.5 mg. per 100 cc.

Phenolsulfonephthalein excretion 25 per cent in 2 hrs.

Temperature 100–102° F.

Blood counts:

June 5. Leucocytes 20,000; polymorphonuclear 80 per cent; lymphocytes 5 per cent; large mononuclear 10 per cent; eosinophils 5 per cent.

Erythrocytes 1,200,000. A few normoblasts, but no megaloblasts were found.

June 10. Erythrocytes 2,400,000.

Patient died in hospital and autopsy revealed the presence of gastric carcinoma.

Experiment 1. June 10, 1920.

CO_2 absorption curve:			
CO_2 tension, *mm. Hg*	82.2	47.9	30.2
CO_2, *vol. per cent*	60.1	51.9	46.0
	61.3	55.1	
CO_2 content of arterial blood, *vol. per cent*			50.9
			50.5
CO_2 " " venous " " " "			52.1
			53.4
Difference between arterial and venous CO_2, *vol. per cent*			2.1
O_2 capacity of blood (24 per cent hemoglobin) *vol. per cent*			4.4
O_2 content of arterial blood, *vol. per cent*			4.8
O_2 " " venous " " " "			0.0
O_2 consumption of blood, *vol. per cent*			4.8
O_2 saturation of arterial blood, *per cent*			100
O_2 " " venous " " "			0
Respiratory quotient of blood			0.44

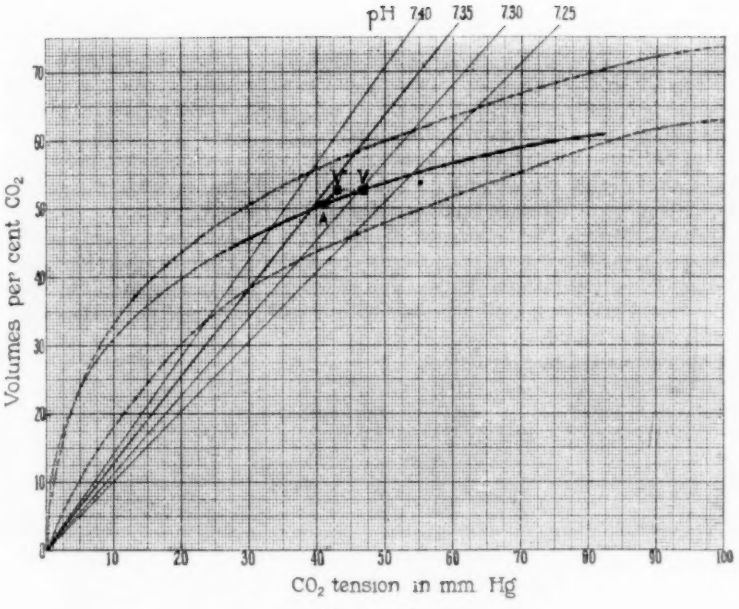
pH 740
735
730
725
Volumes per cent CO_2
CO_2 tension in mm. Hg
10
20
30
40
50
60
70
80
90
100

CHART 14.

CHART 15 AND 16.

Case 15. Jas. M. Severe anemia (probably pernicious). Male, age 62 years, married, fireman. Looks chronically ill. Complains only of pains in the stomach. Mentally sluggish and unable to give any information. Extremely weak. Vomiting greenish brown fluid, streaked with mucus, which contains much bile, 10 per cent acid, no free HCl, and many pus cells. Thin and emaciated, extremely pale. No signs of dyspnea or hyperpnea while at rest in bed. Teeth all gone. Lungs clear. Heart normal. Pulse 58 to 108.

Blood counts:

May 4. Leucocytes 6,200; polymorphonuclear 39 per cent; lymphocytes 59 per cent; large mononuclear 2 per cent; erythrocytes 700,000.

May 6. Leucocytes 2,400; polymorphonuclear 50 per cent; lymphocytes 42 per cent; large mononuclear 2 per cent; erythrocytes 700,000; a few normoblasts and megaloblasts found.

May 8. Leucocytes 3,000; polymorphonuclear 55 per cent; lymphocytes 43 per cent; large mononuclear 2 per cent; erythrocytes 1,900,000; many megaloblasts and a few normoblasts found.

Patient received a transfusion of 630 cc. of citrated blood on May 7. During the night his temperature rose to 103° F., his pulse became very weak, and his respirations shallow and labored. He died at noon the next day. Blood grouping had been carried out with care and no evidence of hemolysis was found.

Experiment 1. May 5, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*.....	39.8	9.1	38.3	78.4
CO_2, *vol. per cent*.....	58.1	45.6	57.7	67.4
	59.7	45.6	57.6	65.9

CO_2 content of arterial blood, *vol. per cent*.....	57.9
	57.3
CO_2 " " venous " " " "	61.1
	61.3
	62.2
Difference between arterial and venous CO_2, *vol. per cent*...	3.9
O_2 capacity of blood, *vol. per cent*.....	3.4
	3.3
O_2 content of arterial blood, *vol. per cent*.....	2.9
O_2 " " venous " " " "	0.5
O_2 consumption, *vol. per cent*.....	2.4
O_2 saturation of arterial blood, *per cent*.....	87
O_2 " " venous " " "	15
Respiratory quotient of blood.....	1.62

Case 16. E. S. Chronic nephritis with severe anemia. Female, age 21 years, married, housewife. Acutely ill. Fairly well developed and nourished. Very pale. Sitting up in bed, with dyspnea, orthopnea, and slight cyanosis. Moderate edema of feet and legs. Lungs show signs of fluid in both pleural cavities. Heart somewhat enlarged, without murmurs. Rate 96. Blood pressure: systolic 190, diastolic 115. Temperature normal.

Urine shows very heavy cloud of albumin, with many casts, many red blood cells, and some pus. Specific gravity 1.006 to 1.011.

Non-protein nitrogen of blood 165 mg. per 100 cc.

Phenolsulfonephthalein excretion about 5 per cent in 2 hrs.

Blood count:

Mar. 2. Leucocytes 19,200; polymorphonuclear 82 per cent; erythrocytes 4,200,000.

Experiment 1. Mar. 3, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	30.5	53.6	6.4
CO_2, *vol. per cent*	44.8	50.7	33.5
		52.1	33.2

CO_2 content of venous blood, *vol. per cent* 46.8
46.8

CO_2 capacity of venous plasma (33.8 mm. Hg), *vol. per cent*.. 49.1

O_2 content of venous blood, *vol. per cent* 3.7
3.7

Experiment 2. Mar. 5.

O_2 capacity of venous blood (35 per cent hemoglobin), *vol. per cent* 6.5

O_2 content of venous blood, *vol. per cent* 3.1

O_2 saturation of venous blood, *per cent* 48

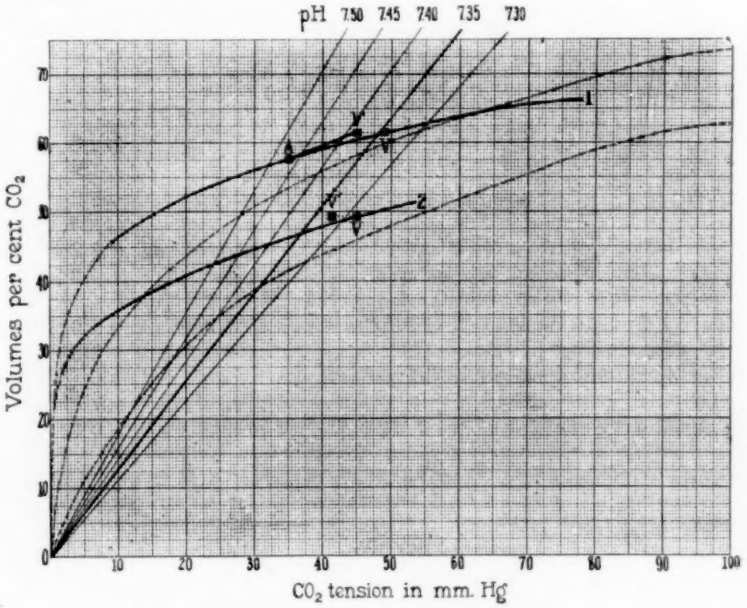

Chart 15 and 16. 1. Case 15.
2. Case 16.

CHART 17.

Case 17. C. P. Pernicious anemia. Male, age 55 years, single, stable worker. Looks chronically ill. Very weak and emaciated. Extremely pale, with lemon-yellow tinge. Some retinal hemorrhages. No signs of dyspnea or hyperpnea while at rest in bed. No cyanosis. Lungs clear. Heart not enlarged. Blowing, systolic murmur at apex. Pulse regular, 90 to 100. Blood pressure: systolic 145, diastolic 60. Temperature 100–102° F.

Blood counts:

May 24. Leucocytes 3,000; polymorphonuclear 50 per cent; lymphocytes 48 per cent; large mononuclear 2 per cent; erythrocytes 700,000. Many normoblasts and megaloblasts found.

June 2. Polymorphonuclear 60 per cent; lymphocytes 25 per cent; large mononuclear 14 per cent; eosinophils 2 per cent; erythrocytes 1,100,000; many normoblasts and megaloblasts found.

Experiment 1. May 28, 1920.

Alveolar CO_2 before arterial puncture, *mm*.			28.3
			27.8
" CO_2 after " " "			28.6
CO_2 absorption curve:			
CO_2 tension, *mm. Hg*.	93.1	46.8	24.0
CO_2, *vol. per cent*.	63.0	55.6	47.3
	64.6	55.9	48.3
CO_2 content of arterial blood, *vol. per cent*.			52.2
			51.6
CO_2 " " venous " " " "			55.2
			54.5
Difference between arterial and venous CO_2, *vol. per cent*.			2.2
O_2 capacity of blood, *vol. per cent*.			4.3
O_2 content of arterial blood, *vol. per cent*.			4.4
O_2 " " venous " " " "			1.6
O_2 consumption, *vol. per cent*.			2.8
O_2 saturation of arterial blood, *per cent*			100
O_2 " " venous " " "			37
Respiratory quotient of blood.			0.79

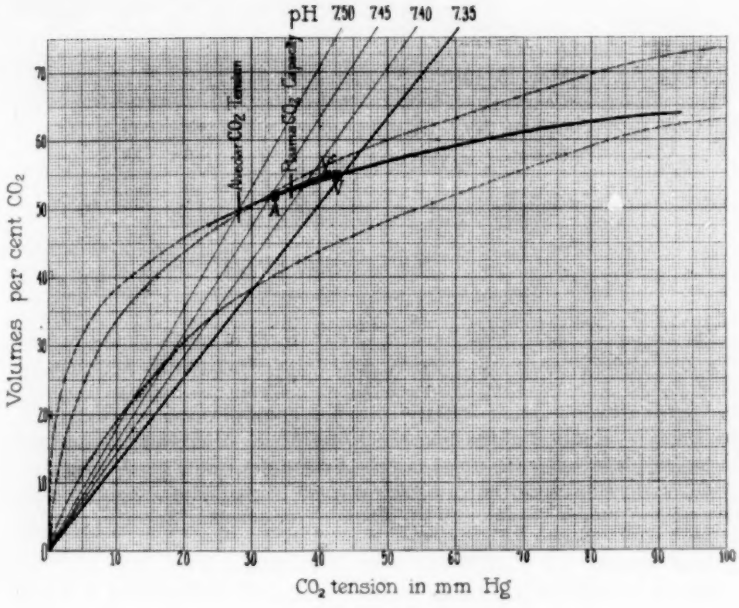

CHART 17.

Additional Protocols.

Case 18. N. B. Pernicious anemia. Female, age 53 years, married, servant. Looks chronically ill. Fairly well nourished and developed, but flabby. Pale, lemon-yellow color. Small hemorrhagic spots in roof of mouth, retinal hemorrhages, and purpuric spots on extremities. No dyspnea or visible hyperpnea while patient is at rest in bed. Lungs clear. Heart slightly enlarged to the left with a soft systolic murmur and accentuation of the pulmonic second sound.

Gastric contents show no free HCl, total acid 25 per cent, no blood. Stools show no gross or occult blood. Gastrointestinal examination by means of x-ray reveals a prepyloric filling defect persisting throughout examination, but no definite evidence of carcinoma.

Temperature varied between 99 and 103. Pulse about 90. Urine showed occasional trace of albumin and a few granular casts; specific gravity 1.006 to 1.015.

Blood counts:

Mar. 11. Leucocytes 5,600; polymorphonuclear 40 per cent; erythrocytes 2,200,000; no nucleated red blood cells.

Mar. 17. Erythrocytes 2,700,000.

Experiment 1. Mar. 15, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	71.9	7.1	42.2
CO_2, *vol. per cent*	63.5	31.6	53.8
	63.2	32.3	53.0

CO_2 content of venous blood, *vol. per cent* 60.1

CO_2 capacity of venous plasma (45.4 mm. Hg), *vol. per cent*.. 66.0

O_2 " " " blood (54 per cent hemoglobin), *vol. per cent* 10.1
9.6

Case 19. A. B. Pernicious anemia. Female, age 47 years, single, housewife. Looks chronically ill. Slightly emaciated, very pallid, with lemon-yellow color. Petechial hemorrhages on hard palate. No signs of dyspnea or hyperpnea while lying quietly in bed. Thyroid distinctly enlarged. Lungs clear. Heart slightly enlarged to left, with soft, systolic murmur at base, not transmitted. Pulse rapid. Spleen not felt.

Gastrointestinal x-ray examination reveals no evidence of new growth. Urine shows moderate amount of albumin, no casts. Specific gravity 1.015 to 1.021. Temperature 99–102° F.

Blood counts:

Mar. 16. Erythrocytes 1,500,000.

Apr. 1. Erythrocytes 1,000,000.

Experiment 1. Mar. 16, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	80.4	46.3	18.0
CO_2, *vol. per cent*	70.0	64.6	51.1
		60.9	

CO_2 content of venous blood, *vol. per cent* 63.8
61.0

CO_2 capacity of venous plasma (47.5 mm. Hg), *vol. per cent*.. 69.1

O_2 " " blood (26 per cent hemoglobin), *vol. per cent*. 6.2
5.8

Case 20. G. A. Gastric carcinoma with anemia.

Experiment 1. Mar. 22, 1920.

CO_2 absorption curve:

CO_2 tension, *mm. Hg*	61.0	25.8	38.8
CO_2, *vol. per cent*	74.6	61.4	69.8

O_2 capacity of blood (53 per cent hemoglobin), *vol. per cent*. 9.9

ON A POSSIBLE ASYMMETRY OF ALIPHATIC DIAZO COMPOUNDS.

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(Received for publication, December 31, 1920.)

In 1915 Levene and La Forge made an observation which pointed to the formation of a diazo compound as an intermediary phase in the process of transformation of glucosaminic acid ethyl ester into anhydro-manonic ethyl ester.[1, 2] This observation brought forth the possibility of asymmetry in the α-carbon atom of the diazo compounds of the sugar acids. Since it was known that also esters of aliphatic α-amino-acids on treatment with nitrous acid were convertible into optically active oxy-acids, and since it was known that under certain conditions the treatment of the amino-acids resulted in the formation of aliphatic diazo esters, it seemed to the authors possible that also in the latter there existed an asymmetry. In 1916, Levene and Senior undertook to test experimentally the existence of optical isomerism in the aliphatic diazo compounds. The work was interrupted because of the conditions of war, Dr. Senior having accepted a commission in the Chemical Warfare Service. The work was resumed in September, 1920, by the present writers. In November there appeared a publication by Marvel and Noyes[3] on the subject of the present article. The experimental results reported by these writers were negative.

Our own work is not yet completed, but in view of the just mentioned publication we wish to record results which point favorably towards the possibility of asymmetry in these diazo compounds.

[1] Levene, P. A., and La Forge, F. B., *J. Biol. Chem.*, 1915, xxi, 345.

[2] Levene, P. A., *J. Biol. Chem.*, 1918, xxxvi, 89.

[3] Marvel, C. S., and Noyes, W. A., *J. Am. Chem. Soc.*, 1920, xlii, 2259.

Dextro aspartic acid was converted into diazosuccinic acid ethyl ester. It was found possible to purify the ester by fractional distillation under a pressure varying from 0.08 to 0.12 mm.

The analytical data of the samples are given in the following table.

	N	Impurity.	$[\alpha]_D^{20}$
	per cent	*per cent*	
Experiment 1.			
First fraction	10.06	30.0	+1.27
Second "	13.68	5	+1.25
Third "	14.10	0	+1.34
Experiment 2.			
First fraction			
Second "	13.64	2.5	
Third "	13.33	5	
Second and third fractions redistilled.			
First fraction	13.76	1.7	+1.27
Second "	13.64	2.5	
Experiment 3.			
First fraction			
Second "	13.64	2.5	+1.00
Third "	14.20	0	+0.85

The theory for $C_8H_{11}O_4N_2$ requires N = 14.02 per cent.

The accuracy of the analytical method was tested on the crystalline diazosuccinic acid monoamide monoethyl ester and on diazoacetic acid ethyl ester.

The impurity of the highest optical activity that could be present in our material is *d*-malic diethyl ester. The optical rotation of this ester is $[\alpha]_D^{20} = +10.18$. It would require the presence of from 8 to 13 per cent of the latter compound if the rotation of our samples were brought about by impurities and not by the diazo compound. Impurities of such proportions could not have escaped detection by the analytical method employed in this work.

On hydrolysis of the diazo esters, optically active material was formed with a magnitude of rotation which did not differ essentially from that of the original material.

The details of the experiments will be reported in a subsequent communication. Further work is in progress.

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